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L18: Entry 32 of 35 File: USPT Jun 11, 1991

US-PAT-NO: 5023090
DOCUMENT-IDENTIFIER: US 5023090 A

TITLE: Topical compositions containing LYCD and other topically active medicinal ingredients for the treatment of ACNE

DATE-ISSUED: June 11, 1991

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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APPL-NO: 07/ 503225 [PALM]
DATE FILED: April 2, 1990

PARENT-CASE:
RELATED APPLICATION The present application is a continuation-in-part of application U.S. Ser. No. 07/394,862 filed 08/16/89, U.S. Pat. No. 4,942,031; which in turn is a continuation-in-part of application U.S. Ser. No. 07/159,390, filed Feb. 23, 1988, the latter application now abandoned, both applications being of the present inventor, Robert H. Levin.

INT-CL: [05] A61K 35/72, A61K 31/70, A61K 33/40

US-CL-ISSUED: 424/520; 514/29, 514/32, 514/725, 514/714
US-CL-CURRENT: 424/520; 514/29, 514/32, 514/714, 514/725

FIELD-OF-SEARCH: 424/520, 514/179, 514/555, 514/570, 514/29, 514/32, 514/480, 514/481, 514/262, 514/50, 514/2, 514/21, 514/297, 514/725, 514/256, 514/714, 514/725, 514/177, 514/178, 514/663

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected Search ALL

	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
☐	2320479	June 1943	Sperti	424/520
☐	4427654	January 1984	Austin	514/520
☐	4797392	January 1989	Chernomorsky	514/185
☐	4814351	March 1989	Mathews et al.	514/566

OTHER PUBLICATIONS

Goodson et al., Journal of Surgical Research 21:125-129 (1976).
Kaplan, Arch Surg 119: 1005-1008 (1984).

ART-UNIT: 188

PRIMARY-EXAMINER: Robinson; Douglas W.

ASSISTANT-EXAMINER: Witz; Jean

ABSTRACT:

A topical composition comprising LYCD together with known topically active useful medicinal agents such as anti-wrinkling, antibiotic, anticancer, antifungal, antiinflammatory such as anti-acne, antiviral, wound healing, and hair-growing agents. The LYCD works together with the other active agents to achieve a synergistic result more effective than can be obtained from the topical agents individually, and more effective than could be predicted from the mere addition of the known efficacies of the individual ingredients.

13 Claims, 0 Drawing figures
Exemplary Claim Number: 1

BRIEF SUMMARY:

1 BACKGROUND OF THE INVENTION

2 1. Field of the Invention

3 The present invention relates to topically applied medicinal compositions, and more particularly refers to such compositions having active topical medicinal ingredients, and additionally having LYCD in amounts sufficient to act with the other active ingredients to provide synergistic therapeutic results.

4 2. Description of the Prior Art

5 LYCD as utilized herein in the specification and claims is the acronym for Live Yeast Cell Derivative. The material is also known as Skin Respiratory Factor (SRF), Tissue Respiratory Factor (TRF), and Procytoxoid (PCO). The product, LYCD, is an alcoholic extract of viable Saccharomyces cerevisiae. The material is produced and marketed by MDH Laboratories, Inc., Cincinnati, Ohio 45210 as a standard article of commerce. Another producer of LYCD is Universal Foods Corporation, Fermentation Division, Milwaukee, Wis. 53202. LYCD is available for experimental use as a bulk drug assaying 5 units to 40 units/mg of respiratory activity. In topical medicinal preparations it is characterized and quantified in terms of Skin Respiratory Factor (SRF) units. A unit of activity is calculated as the amount of SRF which is required to increase the oxygen uptake of 1 mg of dry weight rat abdominal skin by 1 percent at the end of a 1 hour testing period in a Warburg apparatus.

6 LYCD is also available as LYCODERM.RTM. ointment containing 2,000 units Skin Respiratory Factor (SRF) per ounce, from Arel Pharmaceuticals, Inc., Cincinnati, Ohio. In the prior art the well know hemorrhoidal ointment, PREPARATION H.RTM., contains 2000 units of SRF (ca 1%) per ounce of ointment.

7 J. Z. Kaplan (Arch. Surge. 119(9) p. 1005-8 (1984) has reported that, in a double blind human skin graft study donor sites treated with LYCD ointment, statistically significant earlier angiogenesis and epitheliazation occurred as compared with donor sites in the same patients treated with ointment bases (without LYCD). This study confirmed earlier laboratory reports such as that of Wm. Goodson et. al. Journal of Surgical Research 21: 125-129 (1976) showing that LYCD is capable of stimulating wound oxygen consumption, epitheliazation, and collagen synthesis.

8 As reported in the Cincinnati Inquirer of Dec. 12, 1986, Ashlley Hunter Cosmetic Co. offers a facial cream containing LYCD to minimize wrinkles.

9 For milder forms of acne, which may be inflammatory, topical benzoyl peroxide (BP), an antibacterial and oxidizing agent, topical erythromycin (EM), clindamycin phosphate (CP), oral tetracyclines, or EM antibiotics are usually effective treatments, as disclosed in the prior art. (C. D. Bunker, Drugs Today, 24, 229 (1988).

10 SUMMARY OF THE INVENTION

11 It is an object of the present invention to provide topically applied pharmaceutical compositions suitable for the treatment of various ailments and physical conditions of the skin such as acne, bed sores, burns, infections, trauma, ulcers, wounds, and wrinkles.

12 It is a further object to provide compositions of the type described which are more effective than compositions presently known in the art.

13 It is a prime object of the invention to provide topical compositions of the type described for the treatment of acne, and more particularly, severe forms of acne, which compositions are more effective as remedies than the compositions presently known and used in the art.

14 The foregoing and other objects, advantages and characterizing features will become apparent from the following description of certain illustrative embodiments of the invention.

15 According to the invention, pharmaceutical compositions for topical application are provided by utilizing LYCD in combination with known pharmaceutical agents or remedies. The LYCD acts synergistically with the other agents to provide a composition having greater effectiveness than that of the individual agents, and a greater effectiveness than could be predicted by combining (in an additive fashion) the known or theoretical effectiveness of the individual ingredients.

16 According to the invention, it has been further found that many patients with severe acne, refractory to even long term treatment with a variety of the conventional acne remedies experience significant improvement in their acne condition within thirty days when treated twice daily with a combination of any of the conventional antibacterial acne medications and LYCD.

17 It has been additionally found that mild to moderately severe acne can be treated and the condition ameliorated by the application of a topical pharmaceutical composition comprising LYCD in a suitable vehicle, even in the absence of conventional acne remedies.

DETAILED DESCRIPTION:

1 DESCRIPTION OF THE PREFERRED EMBODIMENTS

2 LYCD, Live Yeast Cell Derivative, also known as SRF or TRF, is a commercial material produced by the method set forth in U.S. Pat. Nos. 2,239,345, 2,320,478, and 2,320,479. which are herein incorporated by reference, and is standardized as units with 1 unit (U) of SRF increasing the uptake of oxygen by minced rat abdominal skin (1 mg. dry weight) by 1% in a 1-hour measurement by Warburg manometry.

3 Example 1

4 The compositions of the invention may be produced by either of two general methods. In the first method, for example, an ointment composition may be formulated by mixing LYCD with conventional ointment-forming ingredients. One such ointment composition is LYCODERM.RTM.. a registered trademark material marketed by Arel Pharmaceuticals, Cincinnati, Ohio, having the following compositions:

	Per 100 Parts
Beeswax	4.0
Lanolin	4.0
Petrolatum	87.9
Shark liver oil	3.0
Phenyl mercuric nitrate	0.01
LYCD (2000) SRF units/ounce	1.0 approx.
Thyme oil	0.1

5 Procedure

6 In preparing the above compositions, the beeswax, petrolatum, shark liver oil and phenyl mercuric nitrate are treated together to a temperature of 140 deg. F. in a steam-jacketed s.s. kettle. The materials are mixed until the mixture is uniform. Then the steam is turned off and cooling water is introduced while mixing is continued. When the mixture has reached a temperature of 110 deg. F., the LYCD and thyme oil are added. When the composition becomes uniform, mixing is stopped. Filling may be carried out at temperatures of between 90 and 110 deg. F.

7 In utilizing the first method of producing the compositions of the invention, the LYCODERM.RTM. ointment as produced above but utilizing 200 to 1800 SRF units per ounce of formulation is mixed with the known pharmaceutical agent by a conventional method.

8 The second method of carrying out the present invention comprises mixing bulk LYCD into the conventional pharmaceutical composition.

9 In general, it has been found that effective compositions are formulated using LYCD concentrations in the range of about 0.1% to 0.5% by weight of the composition, constituting, for example, 10% to 50% of that employed in the basic LYCODERM.RTM. ointment formulation. This translates into 200 to 1000 LYCD or SRF units per ounce of combined product. Stated in another way, the new compositions have a LYCD concentration of approximately 0.1% to 0.5% compared with approximately 1.0% LYCD in the basic LYCODERM.RTM. formulation. However, for certain compositions LYCD in the amount of 0.8% of the total composition produces optimum results.

10 Correspondingly, the other active components of the new compositions are found to be synergistically efficacious at concentrations in the range of 5% to 50% of that found to be efficacious in marketed pharmaceutical products.

11 ANTI ACNE COMPOSITIONS

12 Example 2

13 The above-described LYCODERM.RTM. ointment was formulated using 1000 units of S RF per ounce. To this formulation was added sufficient 2% erythromycin to yield an ointment containing 0.5% LYCD and 1% erythromycin. In a controlled clinical trial this composition was equally effective in the topical control of acne vulgaris as a conventional 2% ointment preparation of erythromycin. (An example of such a medicinal preparation, NDA 50-584, Akneymycin Topical Ointment, 2%, is a topical erythromycin accepted by the FDA for topical control of acne vulgaris.)

14 Example 3

15 Alternatively, a marketed anti-acne 2% Erythromycin product was reformulated as follows:

Erythromycin Base	1%	w/v
LYCD	0.5%	w/v
Alcohol	55%	v/v
Oleyl Alcohol	5%	w/v
Perfume	q.s.	
Propylene Glycol	to 100%	

16 Following the same treatment regimen, the 1% Erythromycin solution containing approximately 0.5% LYCD of Example 2 (assaying 1,000 units of SRF per ounce of formulation) was found to be more effective than a similar 2% Erythromycin solution without LYCD in the treatment of Acne grades 2 and 3 (moderate to severe).

17 Example 4

18 In an analogous fashion, Lincomycin Hydrochloride or Clindamycin Phosphate at a concentration of 0.5% is combined with 0.5% LYCD in the LYCODERM.RTM. formulation to provide an ointment as effective in treating common acne as the conventional 1% Clindamycin Phosphate topical medication, and more effective than either agent individually or predictable in their combination.

19 Example 5

20 Ten patients suffering with severe acne completely refractory to months of prior treatment with a variety of conventional acne medications were treated with an admixtured combination of Cleocin T (clindamycin phosphate solution) and LYCODERM.RTM. Ointment. The final formulation contained approximately 0.34% clindamycin and 0.56% LYCD. Each subject applied this formulation twice daily for 30 days with the following results:

21 Patient 1: female, age 16: Refractory to Acromycin, Erythrocin, diet, local treatment. The patient was subsequently treated with the low dose combination product of Example 5, and as a result experienced 80% improvement.

22 Patient 2: male, age 25: Refractory to Achromycin, curattage, Acutane. The patient was then treated with the low dose combination product of Example 5, and as a result experienced 90% improvement.

23 Patient 3: male, age 30: Refractory to proprietary medicines, Erythromycin, Achromycin, Fostex (10% Benzoyl Peroxide). The patient was then treated with the low dose combination product of Example 5, and experienced 50% improvement.

24 Patient 4: female, age 21: Refractory to astringents, Erythromycin, and diet. The patient was then treated with the low dose combination product of Example 5, and experienced 85% improvement.

25 Patient 5: Female, age 18: Refractory to Achromycin, diet, and astringents. The patient was subsequently treated with the low dose formulation of Example 5, and experienced almost 100% improvement.

26 Patient 6: female, age 24: Refractory to Achromycin, curettage, and diet. The patient was subsequently treated with the low dose formulation of Example 5, and experienced 85% improvement.

27 Patient 7: Female, age 24: Refractory to Achromycin, diet, local Rx, and proprietary drugs. The patient was subsequently treated with the low dose

formulation product of Example 5, and experienced 85% improvement.

28 Patient 8: Female, age 29: Refractory to Vitamin A ointment, diet, and Achromycin. The patient was subsequently treated with the low dose formulation of Example 5, and experienced 80% improvement.

29 Patient 9: Male, age 30: Refractory to Achromycin, local Rx, and Acutane: The patient was subsequently treated with the low dose formulation of Example 5, and experienced 80% improvement.

30 Patient 10: Female, age 20: Refractory to Achromycin, and coal tar products. The patient was subsequently treated with the low dose formulation of Example 5, and experienced 85% improvement.

31 Example 6

32 In an analogous fashion topical acne treatment formulations containing 5% or 10% Benzoyl Peroxide anti-acne formulations were admixed with LYCODERM.RTM. ointment to provide lower dose Benzoyl Peroxide/LYCD combination products more effective than the higher concentration of Benzoyl Peroxide conventionally used in the treatment of acne vulgaris. Additionally, desquamation, symptoms of burning, and other side effects were less frequent.

33 Example 7

34 In a further test 1.5 oz. FOSTEX.RTM. 5% Benzoyl Peroxide Gel was admixed with 2 oz. LYCD ointment to provide a formulation containing approximately 2% Benzoyl Peroxide and 0.5% LYCD (assaying approximately 1,000 units of SRF per ounce of formulation). Further 1 oz. of CLEARASIL.RTM., 10% benzoyl peroxide anti-acne cream, was admixed with 4 oz. of LYCODERM.RTM. ointment to provide a formulation containing approximately 2% benzoyl peroxide and 0.8% LYCD. Both compositions are effective in treating mild to severe acne.

35 Example 8

36 A LYCODERM.RTM. composition is prepared comprising containing 1000 units of SRF per ounce (ca 0.5%) and 0.25% of vitamin A acid to provide a topical ointment composition more effective than the retinoic acid by itself in ameliorating the effects of moderate to severe acne.

37 Example 9.

38 The LYCODERM.RTM. formulation was applied for twelve weeks to mild to moderately severe acne afflicted subjects in the absence of conventional drugs used in the treatment of acne. The treatment resulted in a 40% reduction in inflammatory lesions. For clinical testing purposes the same formulation was prepared as a placebo by leaving out the LYCD. During this period the placebo, when applied to similarly afflicted patients proved to be ineffective,

39 Example 10

40 Various protein/peptide growth factors have been used as topical wound-repair agents. It has now been found that Epidermal Growth Factor (EGF), when used in concentrations of 0.0001% in a suitable topical pharmaceutical formulation is an effective treatment for moderate acne.

41 Further, according to the method of the present invention, compositions containing 500 units per ounce of LYCD/SRF incorporated into commercial formulations of recombinant/human Epidermal Growth Factor preparations or their reduction products (containing 0.0001% rh EGF) are synergistically more effective in treating severe acne.

42 FIRST AID, BURN, AND WOUND-HEALING COMPOSITIONS

43 Example 11

44 Several commonly used topical antibiotic preparations are accepted by the FDA for non-prescription (OTC) use to help prevent infection and aid in the healing of minor cuts, burns, and abrasions. One such preparation contains 0.5% neomycin sulfate. The second contains 500 units of bacitracin, 5000 units of polymixin B sulfate, and 0.5% neomycin sulfate per gram of composition. To each of these formulations was added 1000 units of LYCD (approximately 0.5%) per ounce of product. The resulting LYCD antibiotic compositions were synergistically more effective for topical first aid use than the conventional components when used separately.

45 Example 12

46 In an analogous example, a LYCODERM.RTM./antibiotic combination ointment product was formulated as follows:

Polysorbate 80	2	pounds	1.00% w/w
Polymixin B (7,700 iu/mg)	117.02	grams	0.129% w/w
Bacitracin Zinc (690 u/mg)	656.81	grams	0.724 w/w
Phenyl Mercuric Nitrate	9	grams	0.01% w/w
LYCD (12 u/mg)	266.7	grams	0.50% w/w
Deionized water	4# 7	oz	2.50% w/w
Beeswax (white)	8# 1	oz	4.00% w/w
Lanolin	8# 1	oz	4.00% w/w
Petrolatum	167# 13	oz	83.93% w/w
Shark liver oil	6# 1	oz.	3.03% w/w
Thyme oil	90	grams	0.10% w/w

47 Procedure

48 In preparing the above composition, the first four ingredients were mixed and suspended well. The LYCD is dissolved in the water and mixed well with the first ingredients. The resulting solution was heated to 140.degree. F.

49 Separately, the third group of ingredients was mixed in a clean container and heated to 160.degree. F. With good mixing the first solution was added to the contents of the container; the thyme oil was then added and the composition permitted to cool to 60.degree.-80.degree. for filling into containers.

50 The product thus produced is ideal for treating minor cuts, burns, and scratches. It appears to work rapidly and especially well on infants and children, where the ointment formulation functions somewhat like an occlusive dressing.

51 Example 13

52 A similar LYCODERM.RTM./antibiotic ointment formulation was also prepared using LYCD at a concentration of approximately 1.0% (2000 units SRF/ounce of product.

53 Used twice a day for two to four weeks as an emollient in a group of 10 elderly patients suffering from bed sores, this product resulted in 50 to 100% healing.

54 Separately, LYCD or LYCODERM.RTM. ointment may be incorporated into first aid type bandages or dressings to provide a superior product for burn and wound healing.

55 Example 14

56 Topical antiinfective compositions for wounds including burns may be provided utilizing LYCD in combination with silver sulfadiazine and a suitable ointment-forming base, or LYCD in combination with povidone-iodine and a suitable ointment-forming base.

57 Live yeast cell derivative (LYCD) per se increases collagen formation. It is accepted in the art that most agents promoting experimental wound healing appear to act primarily to promote collagen synthesis. Controlled clinical studies of LYCD have demonstrated both clinically and statistically earlier angiogenisis, initiation and completion of epithelialazation, and acceleration of wound healing.

58 The use of a composition such as LYCODERM.RTM. formulated with 2000 units of LYCD per ounce of ointment (about 1.0%), and also containing 3% of shark liver oil has also been shown clinically to promote wound healing, including a range of first, second and third degree burn wounds.

59 Separately, a number of lymphokine/cytokine proteins have been found which enhance wound healing by directly activating macrophages or indirectly stimulating the skin immune system. More than several dozen of these naturally occurring growth factors have been reported in the literature, but are difficult to isolate and characterize, and may actually overlap in identity. Therapeutic doses, although measured in fractions of milligrams, are very costly.

60 Example 15

61 Epidermal Growth Factor (EGF) is one such growth factor which has been used topically at a concentration of 0.0001% to accelerate normal wound healing by 15-20 percent. According to the method of the present invention, compositions containing 500 units of LYCD as SRF (approximately 0.25%) and 0.0001% EGF are synergistically more effective in treating chronic epidermal ulcers. Similarly using LYCD at 0.1% to 0.5% concentrations (equivalent to 200-1000 LYCD units per ounce of product) in compositions with Fibroblast Growth Factor (FGF), Platelet-Derived Growth Factor (PDGF), Transforming Growth Factor-alpha (TGF-alpha), Transforming Growth Factor-beta (TGF-beta), or Insulin-like Growth Factor-1 (IGF-1) has provided synergistic wound healing compositions. Both partial and full incisional wounds show synergistic healing patterns. Compositions consisting of several of these growth factors formulated with LYCD as in LYCODERM.RTM. also result in synergistically acting wound healing products.

62 Example 16

63 In the area of surgical incisions and wounds, as for example in Gastrointestinal Surgery, excess scarring of the tissue is a not uncommon side effect resulting in surgical adhesions which may require a second operation for correction.

64 It has now been found that a 10% sterile isotonic saline solution of LYCD can be used as a final incision lavage in such surgical procedures to minimize scarring during the wound healing process. A range of LYCD concentrations of 1% to 25% may be used; and isotonic lactose as well as sterile water are suitable vehicles for this purpose. Additionally, as previously disclosed herein, the LYCD solution stabilizes other growth factor/lymphokine/cytokine proteins at the 1 to 100 nanogram per ml. concentrations required for therapeutic purposes. Thus, for example, 5 nanograms per ml. each of insulin-like growth factor-1 and platelet derived growth factor are added to the 10% LYCD solution to make a growth factor "cocktail" which, when used as a final lavage, significantly reduces the "adhesion" side effect of many cardiac, neurovascular, and gastrointestinal surgical procedures.

65 Separately as an adjunct to orthopedic surgery, LYCD, formulated as a 5% sterile solution is combined with one or more of a group of growth factors/cytokines (in

concentrations of one to 100 nanograms per ml.) to provide compositions for the acceleration of healing of bone and other hard tissue injury. Representative factors include: Bone Morphogenic Protein, Cartilage-derived Growth Factor, Cartilage Inducement Factor, Connective Tissue Activating Peptide III, Fibroblast Growth Factor-basic, Osteogenic Growth Factor, Osteogenic Protein, Osteogenin, Skeletal Growth Factor, Tissue Inhibitor of Metalloproteinese, Transforming Growth Factor alpha, and Transforming Growth Factor beta.

66 Example 17

67 In another embodiment of this invention lymphokine/cytokine modulating chemicals, such as Tilorone and its congeners, are formulated into topical wound healing compositions in combination with LYCD. Thus the LYCODERM.RTM. formulation previously described is prepared using 1000 units of SRF per ounce (ca 0.5% and 0.1% of Tilorone to produce a synergistically effective ointment for the treatment of severe burn wounds and non-healing epidermal ulcers. Depending on the formulation, Tilorone synergy can be demonstrated at concentrations ranging from 0.01% to 0.5% in epithelial tissue repair experiments.

68 ANTIFUNGAL COMPOSITIONS

69 Example 18

70 Topical antifungal compositions are used in the treatment of cutaneous or mucocutaneous mycotic infections caused by Candida species, pathogenic dermatophytes, other yeasts, and various superficial fungal infections of the skin. Tolnaftate USP at the level of 0.5%, when formulated with LYCODERM.RTM. containing LYCD at a concentration of 1000 units per ounce (ca 0.5%) provides a synergistic composition more effective than the standard topical antifungal preparation containing 1.0% of Tolnaftate. In an analogous manner, Nystatin USP at a concentration of 50,000 units per gram when formulated with LYCD at the 0.5% concentration, and chlortrimazole 0.25% provided compositions equal in antifungal effectiveness with the topical antifungals used alone at 3 and 4 times the concentration employed in the compositions of the invention.

71 Example 19

72 In similar manner 30 cc. of clotrimazole solution, USP 1%, was admixed with 3 oz. of LYCODERM.RTM. ointment, and the resulting combined formulation used to treat a severe tinea pedis infection in a 70 year old male. With twice a day application into the affected and surrounding skin areas, the infection cleared up within a week.

73 ANTIVIRAL COMPOSITIONS

74 Example 20

75 Topical antiviral medicinal agents are licensed by the FDA particularly for the treatment of herpes simplex and herpes genitalis. The agent idoxuridine is marketed as a 0.5% ointment. When formulated as a 0.1% concentration of idoxuridine and 0.5% concentration of LYCD (containing 1000 units SRF per ounce), the topical composition showed synergistic anti-herpes simplex viral activity. Similarly, a topical composition containing 0.5% acyclovir and 0.5% LYCD (containing 1000 units of SRF per ounce of LYCODERM.RTM. ointment formulation) gave better results in the treatment of herpes genitalia than would be anticipated from the known antiherpes activities of acyclovir in the absence of LYCD. The antiviral efficacy of topical formulations of alpha, beta, or gamma interferons (used at doses of 3 million to 10 million IU) per 30 ml. solution are also enhanced synergistically by the addition of 0.1% to 0.5% of LYCD representing 200 to 1000 units of SRF per ounce of topical composition.

76 In laboratory cell culture studies, the effect of LYCD, lymphokines and lymphokine modulating chemicals on vaccinia virus infected monkey kidney BSC-40 cells, or human epidermoid A431 cells was investigated. Vaccinia virus is a DNA

virus, as is herpes.

77 Example 21

78 Pre-treated experiments: Each cell line was pretreated with a candidate antiviral compound for 24 hours, washed, and then infected with low multiplicities of vaccinia. After 24 hours of infection, virus was titered on BSC-40 monolayers. The data is expressed in placque-forming units (PFU); and the results of duplicate experiments averaged.

79 Post-infection experiments: Vaccinia infected cells were exposed to the test material for 24 hours.

80 The 24 hour preincubation of BSC-40 cells with 100 to 200 micrograms per ml. of LYCD, followed by vaccinia virus infection resulted in a 30% to 40% reduction in PFU. Higher concentrations of LYCD did not quantitatively change the viral inhibition.

81 Example 22

82 The immunomodulating drug Tilorone, and two of its analogs, RMI-11567, and RMI-11645 were similarly tested for their ability to induce an antiviral state in BSC-40 cells by pretreatment of the cells for 24 hours. (See Progress in Medicinal Chemistry, vol. 18, pp. 136-190, 1981 for a description of these compounds) In these experiments 2-3 micrograms per ml. of the tilorones resulted in an inhibition of viral growth which peaked at 40%, with RMI 11567 being the most active.

83 Example 23

84 In analogous experiments, pretreatment with the lymphokines gamma interferon, and Interleukin-I (IL-1) at even lower doses (250 units per ml. and 10 units per ml., respectively) resulted in a 60% to 90% stimulation of viral growth. However, combination of each of these materials with 100 micrograms per ml. of LYCD synergistically reduced or reversed this stimulation.

85 Example 24

86 Surprisingly, in Post-infection cell culture experiments, 1 microgram per ml. of RMI-11567 and 100 micrograms per ml. of LYCD each separately caused a 30% stimulation in viral growth; however, the combination of RMI-11567 and LYCD resulted, synergistically, in a 15% inhibition of vaccinia growth as measured by plaque forming units (PFU). The percent stimulation/inhibition was calculated by comparing the PFU to concurrent control experiments which did not contain any drug.

87 Example 25

88 In analogous Post-Infection studies with A431 cells, somewhat similar results were obtained. Thus 8 (standard) units per ml. of alpha or beta Interleukin-I resulted in a slight (3%) inhibition of vaccinia growth as measured by PFU; and 100 micrograms per ml. of LYCD resulted in an 18% inhibition of PFU. The combination of 8 units per ml. of IL-1 and 100 micrograms per ml. of LYCD resulted in a synergistic 32-35% inhibition of viral growth as measured by placque-forming units (PFU).

89 The experiments described in the examples above demonstrate that in cell culture LYCD has significant antiviral activity which can synergistically enhance the antiviral effects of some lymphokines and lymphokine modulating chemicals.

90 As more fully described below, a standardized LYCD preparation assaying 12 units of Skin Respiratory Factor (SRF) per mg. was used in the above described cell culture studies.

91 Example 26

92 The addition of 0.5% fluorouracil to the above-described LYCODERM.RTM. ointment formulated with 1000 units of SRF (about 0.5% LYCD) per ounce of product provides a composition for the topical treatment of multiple actinic (solar) keratoses which is synergistically more effective than a conventional topical preparation formulated with 1.0% fluorouracil.

93 ANTIINFLAMMATORY COMPOSITIONS

94 Example 27

95 Triethanolamine salicylate (10%) is formulated in lotions and creams to provide a topical external analgesic agent for temporary relief from minor pains of arthritis, rheumatism, and muscular aches. When formulated using 5% of triethanolamine salicylate and 200 to 1800 units SRF per ounce of product (equivalent to approximately 0.1% to 0.8% of LYCD), the new composition was synergistically more effective than the original analgesic product containing 10% of triethanolamine salicylate. In an analogous manner, when LYCODERM.RTM. is formulated with non-steroidal antiinflammatory agents such as ibuprofen or its isomers (at a level of 1.0% to 5.0%), the resulting topical compositions have a higher level of topical anti-inflammatory efficacy than that demonstrated by the same preparation of the non-steroidal antiinflammatory agent (NAIA) formulated without LYCD.

96 The adreno-corticoid steroids demonstrate pleitropic activity in cell culture systems and as topical anti-inflammatory agents.

97 Metabolically, LYCD biological activity at the cellular level in animal and human skin results in an increase in oxygen respiration and cell growth. However, in specific human cell lines including human fibroblasts, the effects are variable.

98 Example 28

99 A number of standard human cell lines were evaluated and methodology was developed leading to the use of A431 cells, Am Type Culture Collection CLL 1555, as a suitable human cell line to study the effect of LYCD on oxygen respiration and cell growth. In order to facilitate study of the metabolic interaction of LYCD with lymphokines, cytokines, other growth factors, and topically useful therapeutic agents, it was necessary to achieve reproducible base line results in a defined serum-free cell culture medium. Cell respiration was quantified using a Clark oxygen electrode oxygraph apparatus. Experiments were done in duplicate, and cell number measurements were always done in quadruplicate, for any single experiment, the amount of (oxygen) respiratory stimulation or inhibition of a measured number of A431 cells can be correlated with the concentration of added LYCD. Respiration is measured within 6-10 minutes of adding the LYCD and/or other substrates.

100 A standardized LYCD preparation assaying 12 units of SRF/mg. was used in these studies. In a typical titration of A431 cell respiration it was found that 0.75 to 1.25 mg/ml of LYCD resulted in about a 60% increase in respiration. An LYCD concentration of 0.15 mg/ml gave a 10 to 15% increase in respiration; and 1.50 mg/ml of LYCD resulted in a 20% increase in respiration. Higher concentrations of LYCD resulted in no stimulation of baseline respiration.

101 Example 29

102 In companion experiments it was determined that 25 to 35 picomolar concentrations of hydrocortisone inhibited A431 respiration, but, when added together with LYCD synergistically doubled the respiratory stimulation of 0.2 mg/ml LYCD from 20% to 40%.

103 A431 cell growth experiments were compared at seven days using a standard commercial serum-free medium (Gibco DME, Dulbecco's Minimum Essential media) and ITS (5 micrograms/ml each of insulin, transferin and selenium).

104 It was found that a 0.70 mg/ml of LYCD caused significant and reproducible growth of A431 cells. This concentration of LYCD represents about 12% of the concentration of LYCD found in presently marketed products containing LYCD.

105 LYCD concentrations of 0.02 mg/ml provided a 30% enhancement in A431 cell growth.

106 Hydrocortisone has been reported in the literature to be an inhibitor of A431 cell growth.

107 Example 30

108 A seven day study was made to determine the effect of hydrocortisone on cell growth. It was found that hydrocortisone at the very low concentration of 1.times.10.sup.-8 mg./ml. inhibits A431 cell growth by 65%. However, it was further surprisingly found that the combination of the hydrocortisone plus 0.75 mg/ml of LYCD enhances cell growth by 200%.

109 Example 31

110 A LYCODERM.RTM./hydrocortisone ointment combination product for topical antiinflammatory therapy was formulated as follows:

Ingredient	Amount	% w/w
Polysorbate 80	2 pounds	1.00
Hydrocortisone Acetate	1 pound	0.50
Phenyl Mercuric Nitrate	9.0 grams	0.01
LYCD (12 u/mg)	533.34 grams	1.0
Deionized water	2.25 pounds	2.0
Beeswax (white)	8 lb. 1 oz.	4.04
Lanolin	8 lb. 1 oz.	4.04
Petrolatum	168.5 pounds	84.28
Shark Liver Oil	6 lb. 1 oz.	3.03
Thyme Oil	90 grams	0.10

111 Procedure

112 The first 3 ingredients were combined and mixed well. The LYCD was dissolved in water, combined with the first group and mixed well. The third group of ingredients was added to a clean container, heated to 160.degree. F. with good mixing. With stirring, the water mixture was heated to 140.degree. F. and added to the petrolatum preparation in a container. While stirring continued the mixture was cooled to 100.degree. F. then the thyme oil was added and the mixture further cooled to 60.degree.-80.degree. F. for filling. The formulation thus prepared was found to provide a superior topical antiinflammatory product.

113 Herpes zoster (shingles) is an acute inflammatory disease of the cerebral ganglia and ganglia of the posterior nerve roots, caused by the virus of chicken pox. It is characterized by groups of small vesicles on inflammatory bases occurring in cutaneous areas supplied by certain nerve trunks, and associated

with neuralgic pain.

114 Severe clinical herpes zoster is generally not helped by treatment with presently available antiviral / anti-inflammatory medications.

115 Example 32

116 A number of herpes zoster patients were treated by Sidney Peerless, M.D. of E.N.T. Associates, 3131 Harvey avenue, Cincinnati Ohio 45229. The treatment was carried out after failure of conventional medication, and comprised treatment with a composition according to the present invention comprising the LYCODERM.RTM./Hydrocortisone Acetate formulation shown above in Example 31.

117 Patient 1: This patient had shingles of 4 weeks duration, herpes of the right face and forehead. Symptoms: severe pain, breaking out pustules, and redness. Previous treatment: Zovirex capsules, Zovirex ointment, and antibiotics did not help. The patient was placed on LYCODERM.RTM./Hydrocortisone Acetate ointment of Example 31 applied 2-4 times per day to the affected areas. In 3 days the patient showed marked improvement, especially in the pustules and also in the pain threshold. Within 10 days the lesions were improved and the patient felt much better symptomatically.

118 Patent 2: The patient had severe shingles in the cervical area going into the lower portion of the jaw and into the neck characterized by pustules, severe pain and erythema. Under previous treatment by a dermatologist he had received steroids systemically and Zovirax ointment. After having the disease for three weeks he came to see Dr. Peerless in desperation, because of the severe pain he experienced. The patient also had herpes lesions in his throat. He was placed on the LYCD/Steroid combination ointment of Example 31. After 10 days of treatment the entire facial and neck lesions were gone. There was a marked diminution of pain and need for Demerol, and his general condition improved greatly.

119 Patient 3: The patient had severe shingles involving the right posterior leg and up to the dorsum of the foot. Under the care of another physician the patient had received steroids, antibiotics systemically, and an antibiotic ointment, Polysporin, applied to the lesions without improvement. The patient came to see Dr. Peerless also in desperation. She was placed on the LYCD/steroid combination ointment of Example 31. After three weeks the lesions were almost completely cleared. The pain factor was gone. The patient was able to wear her shoe and showed overall marked improvement.

120 Patient 4: This patient had shingles of the lower lumbar area along the nerve root with large pustules, erythema and almost uncontrollable pain. He had been on pain medicine and Zovirax. He had also been given systemic antibiotics and steroids with very little improvement. The patient was placed on the LYCODERM.RTM./Hydrocortisone Acetate ointment of Example 31. In 48 hours his condition improved markedly, especially in reduction of pain. After another 4-5 days on the medication the herpetic lesions were almost completely under control, and medication was continued for another week. Three weeks after stopping the medication, the patient had a recurrence of the herpes. Readministration of the LYCD/steroid ointment of Example 31 for two weeks again brought the herpes under control, and the patient has remained well.

121 Example 33

122 A formulation was analogously prepared in which the hydrocortisone acetate concentration was reduced to 0.1%. This formulation was found to enhance the anti-erythema, wound-healing properties of the combination product.

123 Example 34

124 Alternatively, LYCD at levels of 200 to 1800 units SRF per ounce (approximately 0.1% to 0.8%) are added to conventional formulations (creams, lotions, ointments, gels, etc.) of compatible topical adrenocorticoid formulations which are used in concentrations of 0.01% to 1.0% to produce synergistic therapeutic

compositions providing more effective medication for the same indications presently approved by the FDA.

125 A representative listing of Topical Adrenocorticoids which may be used in formulating compositions according to the present invention maybe found in the U.S. Pharmacopeial Convention 1986 publication "THE PHYSICIANS' AND PHARMACISTS' GUIDE TO YOUR MEDICINES", published by Ballantine Books, N.Y.N.Y.

126 Example 35

127 More particularly, the addition of 0.25% hydrocortisone acetate to the LYCODERN.RTM. formulation containing 1000 units of LYCD per ounce of ointment (approximately 0.50%) results in a topical antiinflammatory medicinal composition with greater activity and efficacy than is presently available in any topical steroid product licensed by the U.S. FDA for OTC (over the counter) use.

128 ANTI-SKIN WRINKLING COMPOSITIONS

129 Example 36

130 Skin wrinkling is accelerated by deficiencies in collagen synthesis/metabolism. Vitamin A acid (all-trans retinoic acid) is used as a topical preparation to slow or reverse the process of wrinkling. However, its use is limited by concentration related toxicity. According to the present invention LYCD compositions synergistically augment the anti-wrinkling actions of topical retinoic acid with no increase in toxicity. Thus the LYCODERM.RTM. formulation previously described is prepared using 1000 units of SRF per ounce (ca 0.5% LYCD) and 0.25% of vitamin A acid to provide a topical ointment composition synergistically more effective than retinoic acid in ameliorating the skin wrinkling process, including photo-aged skin.

131 Ecample 37

132 Alternatively, presently used cream (0.1%), gel (0.025%), or liquid (0.05%) Vitamin A acid formulations are augmented with 0.5% of LYCD (about 1000 units SRF per ounce to provide synergistically more effective anti-wrinkling compositions. Additionally, these novel compositions are beneficial in treating ichthyosis, actinic keratosis and other hyperkeratotic conditions.

133 LYCD may also be combined with other retinoic acid congeners, known collectively as retinoids, which are also useful topical anti-wrinkling agents to produce new compositions as covered by the present invention. A number of these epidermally acting retinoids are described, for instance, in the special issue supplement to The Journal of the American Academy of Dermatology (Volume 15, No. 4, Part 4, October 1986 entitled "TOPICAL RETINOIDS: AN UPDATE".

134 HAIR GROWTH STIMULATION COMPOSITIONS

135 Few effective agents are currently available for stimulation of hair growth. Only one topical preparation, a 2% solution of minoxidol, manufactured and marketed by the Upjohn Company, Kalamazoo, Mich., is presently approved by the Food and Drug Administration as a pharmaceutical preparation for the treatment of male pattern baldness (alopecia androgenica) of the vertex of the scalp. At least four months of continuous use is generally required before evidence of hair growth can be seen. The historical development of topical treatments for alopecia is more fully set forth in U.S. Pat. Nos. 4,139, 619 and 4,596,812 which are herein incorporated by reference.

136 It is an additional objective of the present invention to provide novel and effective treatments for male pattern baldness by the application of topical pharmaceutical compositions incorporating LYCD at the 0.1% to 3.0% level in pharmaceutically acceptable formulations (solutions, creams, gels, ointments, etc.).

137 Example 38

138 The following LYCD solution was prepared containing 2000 units of SRF per ounce:

LYCD/SRF	1.0%	w/v
Alcohol	55.0%	w/v
Oleyl alcohol	5.0%	w/v
Propylene glycol	to 100%	

139 When applied to the total affected are of the scalp of balding males twice daily for four months, evidence of hair regrowth is observed.

140 Example 39

141 The following cream formulation of LYCD is also useful for promoting hair growth. The cream contains (per 100 grams):

LYCD/SRF	5250	units
Dimethicone	5.0	g
D-pantheol	4.0	g
Benzalkonium Chloride	0.1	g
A water washable cream base	to 100	g

142 Example 40

143 For some individuals, another effective composition for treating male pattern alopecia combines LYCODERM.RTM. ointment and very low dose hydrocortisone acetate, formulated as follows:

Ingredient	per 100 parts by wt.
Polysorbate 80	1.0
Hydrocortisone Acetate	0.1
Phenyl Mercuric Nitrate	0.01
Live Yeast Cell Derivative (LYCD/SRF, 2000 units/oz.) (approximate)	1.0
Deionized water	2.0
Beeswax (white)	4.0
Lanolin	4.0
Petrolatum	84.8
Shark Liver Oil	3.0
Thyme Oil	0.1

144 Procedure

145 The first three ingredients were combined and mixed well. The LYCD was dissolved in water, added to the first group and again mixed well. The third group of ingredients were added separately to a clean container and heated to 160.degree. F. with good mixing. With continued stirring, the water solution was heated to 140.degree. F. and added to the petrolatum/lanolin/beeswax mixture. Stirring continued as the total preparation was allowed to cool to 100.degree. F. Then the thyme oil was stirred in and the mixture further cooled to 60.degree.-80.degree. F. for filling.

146 Example 41

147 Combinations of LYCD with low doses of Minoxidol or retinoic acid also provide synergistic compositions for treating alopecia androgenica. Advantageously, 0.5% of LYCD is formulated with 1.0% minoxidol solution or with 0.1% of vitamin A acid to provide more effective hair growth compositions, although application for four months may still be required before evidence of hair regrowth is observed.

148 COMPOSITIONS FOR USE IN OPHTHALMOLOGY

149 Example 42

150 A 1% to 10% solution of LYCD, in a pharmaceutically acceptable ophthalmic formulation provides a useful composition for the treatment of various eye problems and accelerates corneal epithelial regeneration and the healing of stromal incisions following corneal transplant surgery. More particularly, a 5% solution of LYCD is useful for the acceleration of corneal epithelial regeneration and the healing of stromal tissue in the condition of non-healing corneal defects.

151 Formulated in combination with low doses of a fibronectin or a laminin (one to 100 nanograms per ml.) LYCD is synergistically more effective in treating Dry Eye, Corneal Incisions, Recurrent Corneal Erosions, and Non-healing Corneal Defects.

152 Example 43

153 For the treatment of ocular viral infections, such as, for instance, keratitis due to Herpes simplex virus, the 5% solution of LYCD, in a pharmaceutically acceptable ophthalmic formulation is augmented with an antiviral agent (i.e. 1% trifuridine or acyclovir) and 0.5% hydrocortisone (as its water soluble ester).

154 The compositions of the present invention comprising LYCD in combination with other topically active medicinal ingredients have many advantages over conventional products. The presence of the LYCD in the compositions provides a synergistic effect which makes the conventional materials more effective and permits less of the conventional ingredients to be used while still achieving the same results.

155 The compositions of the present invention contain, in addition to LYCD and the other pharmaceutically active ingredient, a carrier suitable for rendering the composition as a formulation to be used for topical applications. In one method for forming the compositions of the present invention the carrier is provided by the LYCODERM.RTM.. In another method the carrier is provided by the commercial form of the other active ingredient. Alternatively, a suitable carrier known in the art may be added to both the LYCD and the other active ingredient. In all examples above the indicated percent content of the stated ingredients is based on the weight of the total ingredients, the LYCD, the other pharmaceutically active ingredient, and the carrier.

156 The herein described new topical compositions and methods of treatment for a variety of skin conditions are equally applicable to veterinary problems, that is, the treatment of farm animals as well as domestic pets.

157 Although the invention has been described in connection with specific

embodiments thereof, it is evident that many alternatives, modifications, and variations will be apparent to those skilled in the art in the light of the foregoing description. Accordingly, it is intended to embrace all such alternatives, modifications and variations within the spirit and scope of the invention as defined by the appended claims.

CLAIMS:

Invention is claimed as follows:

1. A topical composition adapted for application to the skin comprising in admixture with a pharmaceutically acceptable topical carrier, an antiinfective agent effective in the treatment of acne, and from about 0.1% to about 3.0% by weight of said composition of Live Yeast Cell Derivative (LYCD) in amounts effective to ameliorate the effects of acne when applied to an affected area of the skin.

2. A topical composition according to claim 1, wherein said LYCD is present in an amount of from about 0.1% to about 1.0% by weight of said composition.

3. A topical composition according to claim 2, wherein said antiinfective agent is erythromycin, or a pharmaceutically useful ester thereof.

4. A topical composition according to claim 2, wherein said antiinfective agent is lincomycin hydrochloride.

5. A topical composition according to claim 2, wherein said antiinfective agent is clindamycin phosphate.

6. A topical composition according to claim 2, wherein said antiinfective agent is benzoyl peroxide.

7. A topical composition according to claim 2, wherein said antiinfective agent is vitamin A acid.

8. A method for ameliorating the topical symptoms associated with acne infection in a human being, which comprises topically applying to an area of the skin of said human being where the symptoms are manifested an effective amount of a composition of claim 1.

9. A method according to claim 8, wherein antiinfective agent is erythromycin or a pharmaceutically useful ester thereof.

10. A method according to claim 8, wherein said antiinfective agent is lincomycin hydrochloride.

11. A method according to claim 8, wherein said antiinfective agent is clindamycin phosphate.

12. A method according to claim 8, wherein said antiinfective agent is benzoyl peroxide.

13. A method according to claim 8, wherein said antiinfective agent is vitamin A acid.

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L18: Entry 17 of 35 File: USPT Jul 29, 1997

US-PAT-NO: 5652209
DOCUMENT-IDENTIFIER: US 5652209 A

TITLE: Use of secretory products of human lacrimal gland acinar epithelia for tear replacement therapy

DATE-ISSUED: July 29, 1997

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APPL-NO: 08/ 235673 [PALM]
DATE FILED: April 29, 1994

INT-CL: [06] A61 K 38/18

US-CL-ISSUED: 514/2; 514/12, 514/13, 514/912
US-CL-CURRENT: 514/2; 514/12, 514/13, 514/912

FIELD-OF-SEARCH: 514/2, 514/12, 514/912

PRIOR-ART-DISCLOSED:

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Search Selected | Search ALL

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FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
WO94/01121	January 1994	WO	

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Chemical Abstract 120 : 124917 (1991), Glaser et al.

ART-UNIT: 125

PRIMARY-EXAMINER: Fay; Zohreh

ABSTRACT:

The present invention relates to medicinal compositions and more particularly refers to such compositions for tear replacement therapy having products of human lacrimal gland acinar epithelia, and more specifically, growth factors or cytokines, in particular, the transforming growth factor beta (TGF.beta.).

4 Claims, 19 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 10

BRIEF SUMMARY:

1 BACKGROUND OF THE INVENTION

2 1. Field of the Invention

3 The present invention relates to medicinal compositions and more particularly refers to such compositions for tear replacement therapy having products of human lacrimal gland acinar epithelia, and more specifically, growth factors or cytokines, in particular, the transforming growth factor beta (TGF.beta.).

4 2. Background Information

5 Aqueous tear deficiency is a common condition that in its most severe form may be associated with disabling ocular irritation, and visual morbidity due to corneal epitheliopathy and/or ulceration. The conjunctival pathology of Sjogren's Syndrome (SS), the most severe type of aqueous tear deficiency, consists of abnormal terminal differentiation with significantly reduced bulbar goblet cell densities (Pflugfelder, S. C. et al. Ophthalmology 1990;97:985-991),

decreased expression of mucins by the superficial epithelium (Table I)(Pflugfelder, S. C. et al. 1994 ARVO abstracts. Invest. Ophthalmol. Vis. Sci. 1994; 34: 1692)), and aberrant expression of immune activation markers (HLA Class II antigens and ICAM I) and interleukin 6 (IL-6) (Jones, D. T. et al. Invest. Ophthalmol. Vis. Sci. (in press)).

TABLE I

Results of Immunohistochemical Staining of Bulbar Conjunctival Epithelial Cells on Impression Cytology Specimens using Mucin-Specific Antibody L6

Group	Temporal Conjuctiva (% +)	Inferior Conjuctiva (% +)
Sjogren Syndrome (SS) ATD	18.2	18.2
Non Sjogren Syndrome ATD	66.7	88.9
inflammatory MGD	77.8	88.9
Atrophic MGD	77.8	100
Control	100	100
	SS vs Inflam. MGD p = 0.022	SS vs non SS ATD p = 0.005
	SS vs Atrophic MGD p = 0.022	SS vs Inflam. MGD p = 0.005
	SS vs control p = 0.001	SS vs Atrophic MGD p = 0.001
		SS vs control p = 0.001

ATD = aqueous tear deficiency, MGD = meibomian gland disease

6 At the present time, biological activity of tears on the health and differentiation of the ocular surface epithelia has not been evaluated. Clinical signs and ocular surface pathologic changes in patients with aqueous tear deficiency suggest that the tears may have more than a lubricating role for the ocular surface. One of the most specific clinical signs of severe aqueous tear deficiency is staining of the conjunctival and/or cornea with the diagnostic dye rose-bengal. Recently reported experimental evidence suggests that rose-bengal staining of the ocular surface epithelia may result from lack of cell coating by normal tear constituents, predominantly tear mucins (Feenstra, R. P. and Tseng, S. C. G. Arch. Ophthalmol. 1992;110:984-993). Mucin-producing goblet cells and production of cell-membrane associated mucins by the superficial stratified epithelia are markers of terminal differentiation in the normal human conjunctiva. A marked reduction in expression of both types of conjunctival mucin has been detected in the conjunctival epithelia of Sjogren's Syndrome patients (Pflugfelder, S. C. et al. Ophthalmology 1990;97:985-991. Pflugfelder, S. C. et al., 1994 ARVO abstracts Invest. Ophthalmol. Vis. Sci. 1994; 34: 1692).

7 Although this may be due in part to mechanical trauma related to the reduced preocular tear film, it may also represent abnormal terminal differentiation due to lack of biologically active tear constituents. At the present time, epidermal growth factor (EGF) is the only cytokine that has been detected in human tears (van Seten, G. B. et al. Graeffe's Arch. Clin. Exp. Ophthalmol. 1989;227: 184-187). Reduced tear EGF concentrations have been reported in one patient with aqueous tear deficiency (van Seten, G. B. et al. Curr. Eye Res. 1991; 10:523-527; however, the biologic activity of tear EGF has not been evaluated.

8 Tear secretion by the human lacrimal gland is influenced by neurotransmitters and hormones (Dartt, D. Curr. Eye Res. 1989;8:619-636; Sullivan, D. A. The Neuro Endocrine-immune Network S. Freier, Editor. Boca Raton, Fla. 1990 CRC Press, pp 199-238). Jordan and Baum have reported that the majority of tear secretion is

reflexive, resulting from sensory stimulation of the lids and ocular surface (Jordan, A. and Baum, J. Ophthalmology 1980;87:920-930). A marked reduction in neural-stimulated tear secretion is an early clinical sign in Sjogren's Syndrome (Tsubota, K. Am. J. Ophthalmol. 1991; 111: 106-108), but the clinical consequences of reduced neural-stimulated tears have not been established.

9 We recently discovered that the pathologic changes associated with Sjogren's Syndrome may be due in part to reduced concentrations of cytokines produced by the lacrimal gland and secreted into the tears that are essential for normal health and differentiation of the ocular surface epithelia. Based on its ability to induce differentiation of intestinal mucosa (Kurokowa, M. et al., Biochem. Biophys. Comm. 1987; 142:775-782), and corneal epithelia (Kruse, F. E. and Tseng. S. C. G. Invest. Ophthalmol. Vis. Sci. 1993;34: 1963-1976), and its ability to down regulate HLA Class II antigen and IL-6 expression (Lucas, C. et al. Ciba Foundation 1991; 157:98-114), we hypothesized that transforming growth factor beta (TGF.beta.) may be one of the biologically essential tear cytokines. Recently, TGF has been reported to be produced by mammary gland acini (Maier, R. et al. Mol. Cell. Endocrinol. 1991;82: 192-198) and secreted into milk.

10 TGF.beta. is a multi-functional biologically essential cytokine. TGF.beta. has a spectrum of biologic activity and has been reported to induce differentiation and inhibit proliferation of mucosal epithelia, including rabbit corneal epithelia (Kurokowa, M. et al. Biochem. Biophys. Comm. 1987; 142:775-782; Kruse, F. E. and Tseng, S. C. G. Invest. Ophthalmol. Vis. Sci. 1993;34: 1963-1976). TGF.beta. has also been reported to stimulate synthesis of extra cellular matrix components and has been shown to induce these effects on corneal stromal fibroblasts (Ohji, M. et al. Curr. Eye. Res. 1993;12:703-709). Finally, TGF.beta. has immunosuppressive activity that includes inhibition of T-cell proliferation, down regulation of expression of inflammatory cytokines such as IL-6 and immune activation markers such as HLA class II antigens (Lucas, C. et al. Ciba Foundation 1991; 157:98-114).

11 At the present time, commercially available artificial tear replacements are composed of synthetic polymers, buffers, and electrolytes in an aqueous solution. Examples of such solutions include "BION" (Alcon Laboratories, Fort Worth, Tex.) and "REFRESH PLUS" (Allersan, Irvine, Calif.). Major components of commercially available artificial tear replacement solutions, Ophthalmic lubricants which protect the eye from drying, and ocular decongestants, are listed in TABLES II, III, and IV, respectively. These solutions contain no biologically active components to modulate the health and differentiation of ocular surface epithelia. Tear replacement therapies containing biologically active components could potentially reverse pathologic ocular surface epithelial changes, and would present a great advance in treatment of severe aqueous tear deficiency states.

TABLE II

ARTIFICIAL TEAR PREPARATIONS MAJOR COMPONENT	CONCENTRATION	TRADENAME	PRESERVATIVE/EDTA
Carboxy methycellulose	0.5%	Cellufresh	None
	1%	Celluvisc	None
Hydroxyethyl cellulose		Lyteers	Benzalkonium Cl + EDTA
		TearGard	Sorbic Acid + EDTA
Hydroxyethyl cellulose + Polyvinyl Alcohol		Neo-Tears	Thimerosal + EDTA

Pydroxyethyl cellulose + Adsorbotear			
			Thimerosal + EDTA
Povidone			
Hydroxypropyl Cellulose		Lacrisert (Biode-	
			None
		gradable insert)	
Hydroxypropyl Methylcellulose			
	0.5%	Isopto Plain	
			Benzalkonium Cl
		Isopto Tears	
			Benzalkonium Cl
		Tearisol	Benzalkonium Cl + EDTA
	1%	Isopto Alkaline	
			Benzalkonium Cl
		Ultra Tears	
			Benzalkonium Cl
Hydroxypropyl Methylcellulose +			
		Tears Naturale	
			Benzalkonium Cl + EDTA
Dextran 70		Tears Naturale II	
			Polyquad
		Tears Naturale Free	
			None
Hydroxypropyl Methylcellulose +			
		Lacril	Chlorobutanol +
Gelatin A			Polysorbate 80
Methylcellulos 1%		Murocel	Methyl- + Propylparabens
Polyvinyl Alcohol			
	1.4%	Akwa Tears	
			Benzalkonium Cl + EDTA
		Just Tears	
			Benzalkonium Cl + EDTA
		Liquifilm Tears	
			Chlorobutanol
	3%	Liquifilm Forte	
			Thimerosal + EDTA
Polyvinyl Alcohol +			
	1%	Hypotears	
			Benzalkonium Cl + EDTA
PEG-400 + Dextose		Hypotears PF	
			EDTA
Polyvinyl Alcohol +			
	1.4%	Murine	Benzalkonium Cl + EDTA
Povidone	0.6%	Refresh	None
		Tears Plus	
			Chlorobutanol

TABLE III

OPHTHALMIC LUBRICANTS	
TRADE NAME	COMPOSITION
AKWA Tears Ointment (Akorn)	Sterile ointment containing white petrolatum, liquid lanolin, and mineral oil.
Duolube (Bausch & Lomb)	Sterile ointment containing white petrolatum and mineral oil.
Duratears Naturale (Alcon)	

Sterile ointment containing white petrolatum, liquid lanolin, and mineral oil.

HypoTears (Iolab)
Sterile ointment containing white petrolatum and light mineral oil.

Lacri-Lube S.O.P. (Allergan)
Sterile ointment containing 42.5% mineral oil, 55% white petrolatum, lanolin alcohol, and chlorobutanol.

Refresh P.M. (Allergan)
Sterile ointment containing 41.5% mineral oil, 55% white petrolatum, petrolatum, and lanolin alcohol.

TABLE IV

DRUG	TRADE NAME	ADDITIONAL COMPONENTS
OCULAR DECONGESTANTS		
Naphazoline Hydrochloride	AK-Con*	Benzalkonium Cl + edetate disodium
	Albalon*	Benzalkonium Cl + edetate disodium
	Clear Eyes	Benzalkonium Cl + edetate disodium
	Degest 2	Benzalkonium Cl + edetate disodium
	Naphcon*	Benzalkonium Cl + edetate disodium
	Opcon*	Benzalkonium Cl + edetate disodium
	Vasoclear	Benzalkonium Cl + edetate disodium
	Vasocon Regular*	Phenylmercuric acetate
Phenylephrine Hydrochloride	AK-Nefrin	Benzalkonium Cl + edetate disodium
	Efricel	Benzalkonium Cl + edetate disodium
	Eye Cool	Thimerosal + edetate disodium
	Isopto Frin	Benzalkonium Cl + edetate disodium
	Prefin Liquifilm	Benzalkonium Cl + edetate disodium
	Relief	--
	Tear-Efrin	Benzalkonium Cl + edetate disodium
	Velva-Kleen	Thimerosal + edetate disodium
Tetrahydrozoline Hydrochloride	Collyrium	Benzalkonium Cl + edetate disodium
	Murine Plus	Benzalkonium Cl + edetate disodium
	Soothe*	Benzalkonium Cl + edetate disodium
	Tetracon	Benzalkonium Cl + edetate disodium
	Visine	Benzalkonium Cl + edetate disodium
DECONGESTANT/ASTRINGENT COMBINATIONS		
Naphazoline Hydrochloride plus Zinc Sulfate	Clear Eyes ACR (Allergy/Cold Relief)	Benzalkonium Cl + edetate disodium
Phenylephrine	Prefrin-Z	

	Thimerosal
Hydrochloride Zincfrin	Benzalkonium Cl
plus Zinc Sulfate	
Tetrahydrozoline	
Visine A.C.	
	Benzalkonium Cl + edetate disodium
plus Zinc Sulfate	

*Prescription medication

12 SUMMARY OF THE INVENTION

13 We have recently been able to culture human lacrimal gland acinar epithelia which secrete proteins typically produced by lacrimal gland secretory acini in vivo (Yoshino, K. et al., Proceedings of the Fourth International Symposium on Sjorgren's Syndrome (1993), p. 804). In addition, we have evaluated human tears for TGF.beta. using the CCL-64 mink lung epithelial cell (MLEC) growth inhibition assay and sELISA. Results indicate that human lacrimal gland acini produce and secrete TGF.beta. into the tears, and that there are factors in human tears capable of binding TGF.beta..

14 It is therefore an object of the present invention to provide cultured human lacrimal gland acinar epithelia as a model of in vivo secretory acinar function. These cultures can be used for testing of agents which stimulate or inhibit tear secretion and the analysis of biologically active tear constituents that are secreted by the lacrimal gland which can be used for the treatment of diseases affecting the ocular epithelia. Specifically, diseases of the ocular surface associated with aqueous tear deficiency.

15 It is another object of the present invention to provide a medicinal formulation suitable for the treatment of various conditions which result in tear deficiency or ocular irritation. Conditions benefiting from physiologic tear replacement include patients with lacrimal gland dysfunction, destruction or surgical removal (Sjogren's Syndrome, post radiation, altered innervation, surgical removal for treatment of tumor).

16 It is yet another object of the invention to provide tear replacement compositions containing TGF.beta. which are more effective than the composition presently in use which do not contain biologically active components. According to the present invention, tear replacement compositions are provided by adding TGF.beta. to a pharmaceutical composition for application to the eye in order to lubricate the eye or to supplement tears.

17 According to the present invention, tear replacement compositions as stated above may also contain any or other components produced by lacrimal gland epithelia, naturally present in human tears such as antimicrobial proteins (for example lactoferrin and lysozyme), retinol binding protein (for example tear specific pre-albumin), biologically active components or cytokines such as epidermal growth factor, or retinol.

18 Compositions according to the present invention can be used to treat aqueous tear deficiency and conditions associated with alterations of the ocular suface epithelia including hyperproliferation, squamous metaplasia, loss of goblet cells, and abnormal terminal differentiation among other ocular surface pathologic changes that lead to ocular irritation.

19 The foregoing and other objects, advantages and characterizing features of this invention will become apparent from the following description of certain illustrative embodiments of the invention.

DRAWING DESCRIPTION:

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A. Expression of TGF.beta.1 mRNA in normal human lacrimal gland biopsies and cultured human lacrimal gland acinar epithelia. PCR products of the appropriate size (161 bp) from amplification of cDNA prepared from human lacrimal gland epithelial cultures (lane 1) and human lacrimal gland biopsies (lane 2-4) with TGF-.beta.1 specific primers were noted on an ethidium bromide stained agarose gel (upper figure) and Southern hybridization (bottom figure). Lane 5 contains TGF-.beta.1 cDNA. Lane 6--blank, Lane 7--molecular weight standards.

FIG. 1B. Expression of TGF.beta.2 mRNA in normal human lacrimal gland biopsies and cultured human lacrimal gland acinar epithelia. A PCR product of approximately 450 bp was noted on an ethidium bromide stained agarose gel of amplification of cDNA prepared from human lacrimal gland epithelial cultures (lane 1) and human lacrimal gland biopsies (lanes 2-4) with TGF-.beta.2 specific primers. On Southern hybridization, three hybridizations signals with approximate sized of 350-, 450-, and 500-bp were obtained from cDNA prepared from cultured lacrimal gland epithelia (lane 1) and one lacrimal gland biopsy (lane 2), while only two hybridization bands (350 and 450 bps) were obtained from cDNA prepared from the other two lacrimal gland biopsies (lanes 3 and 4). Multiple sized PCR products are most likely due to alternate splicing of the region of the TGF-.beta.2 gene amplified with these primers. Lane 5 contains TGF-.beta.2 cDNA. Lane 6--blank, Lane 7--molecular weight standards.

FIG. 2A. Expression of TGF.beta.1 and TGF2 protein in normal human lacrimal gland biopsies. (a) majority of tubuloacinar structures in all five human lacrimal gland biopsies showed immunoreactivity to a polyclonal antibody to all isotypes of TGF-.beta. (pan TGF-.beta. Ab, 40.times. original magnification). (b) Absence of immunoreactivity to TGF-.beta.2-specific antisera was noted in all lacrimal gland biopsies (400.times. original magnification). (c) and (d). TGF-.beta.1-specific antibodies produced strong immunoreactivity with epithelial cells in four or five lacrimal gland biopsies. The strongest staining with TGF-.beta.1 antibodies was noted in the apical secretory portion and lumens of acinar epithelial complexes ((c)--100.times., (d)--100.times. original magnification). (e) and (f). In sections where entire Tubuloacinar structures were visualized (asterisk), TGF-.beta.1 staining appeared stronger in acinar than ductal epithelia ((e) imunofluorescent staining, (f)--phase, 100.times. original magnification).

FIG. 2B. Expression of TGF.beta.1 and TGF.beta.2 protein in cultured human lacrimal gland acinar epithelia. The cytoplasm of cultured human lacrimal gland epithelia stained with both TGF-.beta.1 (top figure) and TGF-.beta.2 (bottom figure) antisera (100.times. original magnification).

FIG. 3. Results of ELISA for TGF-.beta.1 and TGF-.beta.2 in supernatants (spnt) from human lacrimal gland acinar epithelial cultures and control media. TGF-.beta.1 [] in culture supernatants were significantly greater than media or TGF-.beta.2 (*0.169 ng/ml.+-.0.021)in culture supernatants ($P<0.05$)

FIG. 4. Growth inhibitory effects of native human tears in mink lung epithelial cell bioassay.

FIG. 5. Concentration of TGF.beta. in native tears treated with various physicochemical techniques.

FIG. 6. Growth inhibitory effects of human tears following acidification or treatment with n-acetylcysteine ("MUCOSIL.TM.", DEY Laboratories, Napa, Calif.) and heating.

FIG. 7. Effect of TGF.beta. isotype specific neutralizing antisera on antiproliferative effects of human tears.

FIG. 8. Results of ELISA for TGF-.beta.1 and TGF-.beta.2 for human tears. TGF-.beta. concentration in tears is 0.521 ng/ml+0.321. Tear TGF-.beta.1 concentrations were significantly greater than TGF-.beta.2 ($P<0.05$).

FIG. 9. Western blot of native tears treated with n-acetylcysteine and heating,

showing pro-TGF-.beta. binding to high MW complexes (about 1000 kD, probably mucins), and monomeric TGF-.beta.. Lane 1 purified TGF-.beta.1. (R&D), monomer band is present at approximately 12.5 kD (arrowhead); Lane 2. blank; Lane 3. native tears--a high molecular weight band (approximately 100 kD asterisk) is noted; lanes 4-6: tears treated with n-acetylcysteine and heating (lane 4), acidification with HCl (lane 5), and acidification followed by reduction with DTT (lane 6). Two bands of immunoreactivity were noted with these specimens, a stronger band at approximately 110 kD, the size of the pro-TGF-.beta. complex (LAP plus cytokine, star) and a weaker band of the same size as monomeric TGF-.beta. (approximately 12.5 kD. arrowhead)

DETAILED DESCRIPTION:

1 DETAILED DESCRIPTION OF THE INVENTION

2 In accordance with the present invention, tear replacement compositions containing TGF.beta., where TGF.beta. is either TGF-.beta.1 or TGF-.beta.2 or a combination thereof, by way of non limiting illustration, be applied to the eye in animals and humans as a drop or within ointments, gels, liposomes, or biocompatible polymer discs or pellet. They can be attached to, carried by and/or contained within contact lenses that are placed on the eye. In general, it is desired that the mode of application be such that the composition enters the tear film or otherwise makes contact with the surface of the eye.

3 Further in accordance with the invention, a replacement tear composition is made by combining TGF.beta. with a physiologically acceptable carrier. Preferably, the preparation will be unit dose, refrigerated, with or without preservative. The composition may also contain a physiologically compatible ophthalmic vehicle as those skilled in the art can select using conventional criteria. The vehicles may be selected from the known ophthalmic vehicles which include but are not limited to water, polyethers such as polyethylene glycol 400, polyvinyls such as polyvinyl alcohol, povidone, cellulose derivatives such as carboxy methylcellulose, methylcellulose and hydroxypropyl methylcellulose, petroleum derivatives such as mineral oil and white petrolatum, animal fats such as lanolin, vegetable fats such as peanut oil, polymers of acrylic acid such as carboxylpolymethylene gel, polysaccharides such as dextrans and glycosaminoglycans such as sodium hyaluronate and salts such as sodium chloride and potassium chloride, calcium chloride, magnesium chloride, zinc chloride, and buffer such as sodium bicarbonate or sodium lactate. High molecular weight molecules can also be used, such as mucins.

4 Preferred preservatives are physiologically compatible and do not inactivate TGF.beta. or other peptides or cytokines present in the composition. Preservatives include but are not limited to alcohols such as chlorobutanol, and benzalknonium Cl and EDTA, though other appropriate preservatives known to those skilled in the art may be used.

5 In a preferred embodiment, the concentration of TGF.beta. in the tear solution is from 250 pg/ml to 12.5 ng/ml, preferably 200 pg/ml to 12.0 ng/ml. Active TGF.beta. concentrations in human tears range from 250 pg/ml to 12.5 ng/ml (mean 3.83 ng/ml). There appears to be a total latent TGF.beta. concentration of approximately 30 ng/ml in tears. Ideally, therapeutic TGF.beta. should be administered bound to its natural carrier or binding protein(s) in tears. At the present time, these appear to be mucins because immunoreactivity of TGF.beta. in native tears is at a high molecular weight (approximately 1000 kD), the molecular weight of tear mucins. Data suggests that most TGF.beta. in tears is in the proform (approximately 110 kD). Typically, this proform is converted to the active from by proteolytic enzymes such as plasmin. Plasminogen activator is normally found in human tears. It is likely that concentrations of this protein are reduced in patients with aqueous tear deficiency. Therefore, it may be necessary to use purified (lyophilized) active TGF.beta.. The source of this cytokine is not essential. It could be purified from platelets (a rich source of TGF.beta.1) or recombinant TGF.beta. could be used. Alternatively, cultured lacrimal gland acini could serve as the source of TGF.beta.. Other lacrimal gland produced tear constituents which may be desirable to add to physiologic

tear replacements include, lactoferrin, 1-3 g/L (Kijlstra, A. et al. (1983), Br. J. Ophthalmol. 67:199-202), lysozyme, 0.5-4.5 g/L, and Tear specific pre-albumin, 0.5-1.5 g/L (Berman, E. R. Biochemistry of the Eye, Ed. C. Blakemore, Plenum Press, New York, 1991), mucins, and epidermal growth factor (EGF) 0.75-9.7 ng/ml (van Setten, G. B. et al. (1989) Graeffe's Arch. Clin. Exp. Ophthalmol. 22:184-187; Ohashi, Y. et al. (1989) Invest. Ophthalmol. Vis. Sci. 30:1879-1882), and Vitamin A, 16 ng/ml of retinol (Vitamin A is present in tears as retinol but would need to be added to tear replacement as trans retinoic acid) (Ubels, J. L. and Mac Rae, S. M. (1984) Current Eye Res. 3:815-822).

6 The following examples are presented to illustrate further various aspects of the present invention, but are not intended to limit the scope of the invention in any respect.

7 EXAMPLE 1

8 Production of TGF.beta. by Human Lacrimal Gland Epithella

9 We have recently evaluated normal human lacrimal gland biopsies and cultured human lacrimal gland acinar epithelia (Yoshino, K. et al. Sjorgren's Syndrome--Proceedings of the Fourth International Symposium, 1993. Ed. M. Homma, S. Sugai, T. Tojo, N. Miyasajka and M. Akizuki, Kugler Publications, 1994, Amsterdam/New York) for expression of TGF.beta.1 and TGF.beta.2 mRNA and protein using RT-PCR, sELISA and immunohistochemisty, techniques known in the art (Ji, Z. et al. Invest. Ophthalmol. Vis. Sci. (1994 ARVO abstracts) 1994; 34: 1792). TGF.beta.1 and .beta.2 mRNA expression was found in both lacrimal gland biopsies and acinar cultures (FIGS. 1A and 1B). In lacrimal gland biopsies, immunoreactivity to TGF.beta.1 but not TGF-.beta.2 was detected in the secretory portion of the lacrimal gland acinar epithelia adjacent to the lumen by immunohistochemistry (FIG. 2A). The cytoplasm of cultured acinar epithelia showed immunoreactivity to both TGF.beta.1 and .beta.2 specific antisera (FIG. 2B). TGF.beta.1 was detected in supernatants of lacrimal gland acinar cultures in significantly greater concentrations (0.5-2 ng/ml) than the control (culture media on substrate) by sandwich ELISA (sELISA, FIG. 3). Furthermore, stimulation of cultured human lacrimal gland acini with 0.01 mM carbachol (a cholinergic agonist) resulted in at least a 30% increase in TGF.beta.1 concentrations in the supernatants. These experiments indicate that TGF.beta. is produced and secreted by human lacrimal gland acinar epithelia, and that this secretion may be enhanced by cholinergic stimulation.

10 EXAMPLE 2

11 TGF.beta. in Human Tears

12 We recently evaluated human tears for TGF.beta. using the CCL-64 mink lung epithelial cell (MLEC) growth inhibition assay, a conventional assay for the detection of TGF.beta., and sELISA (Danielpour D. et al. (1989) Cell Physiol. 138:79-86). Native human tears produced an anti-proliferative effect in the MLEC assay; however, a flat growth inhibition curve with rapid loss of anti proliferative activity after 3 to 7 serial dilutions was noted with native tears (FIG. 4). Heating and acidification, two physicochemical techniques previously reported to activate latent TGF.beta. increased the concentration of TGF.beta. in human tears calculated at the midpoint of the growth inhibition curves (FIG. 5). Furthermore, incubation of human tears with n-acetylcysteine ("MUCOCIL".TM., DEY Laboratories, Napa, Calif.), a mucolytic and reducing agent, followed by heating at 80.degree. C. for 8 minutes appeared to release latent TGF.beta. in tear samples, compared to tears treated by heating alone (FIG. 6). Following this treatment, a growth inhibition curve with a slow decay of the growth inhibition activity as tear specimens were serially diluted was obtained that resembled the curve obtained with serially diluted purified human platelet TGF.beta.1 (FIG. 6). The anti-proliferative effect of human tears in the MLEC assay could be inhibited by pre-incubation with TGF.beta.1 neutralizing anti-sera but not by TGF.beta.2-specific antisera (FIG. 7).

13 The presence of TGF.beta. in human tears was confirmed by TGF.beta.1 sELISA.

TGF.beta.1 was not detected in native tear samples by sELISA; however, pre-treatment of human tears with n-acetylcysteine followed by heating resulted in an average detectable tear TGF.beta.1 concentrations of 45 ng/ml (range 19.99-67.7 ng/ml). TGF.beta.2 was detected in human tears by sELISA at very low concentrations (521 pg/ml with a range of 316-891 pg/ml) compared to TGF.beta.1 ($p<0.05$).

14 SDS-PAGE and immunoblotting experiments were performed to confirm the molecular weights (MW) of TFG.beta. complexes in human tears.

15 EXAMPLE 3

16 Western Blot Analysis

17 Western blots were preformed as follows. Kaleidoscope pre-stained molecular weight standard was purchased from Bio-Rad (Richmond, Calif.). Human platelet TGF.beta.1, rabbit anti-pan isotype TGF.beta. was purchased from R&D Systems, Inc. Anti-rabbit and anti-goat IgG-POD were purchased from Boehringer Mannheim (Indianapolis, Ind.).

18 Fresh human tear specimens were activated by the following methods: (1) heating at 80.degree. C. for 7 minutes and immediately placed on ice; (2) diluted 1:1 with 10% N-acetylcysteine "MUCOCIL".TM., DEY Laboratories, Napa, Calif.) then heated at 80.degree. C. for 7 minutes and immediately placed on ice, (3) acidification by adjusting pH to 2 with 1N HCl and incubating at room temperature for 1 hour. The pH was then neutralized with one NaOH, (4) acidification, then reduction by addition of 5 ul of 1M dithiothreitol (DTT). All activated specimens were then added to 2.times. sample buffer and boiled at 100.degree. C. for 3 minutes.

19 Mini-protein II 4-20% Ready gels were used for SDSpolyacrylamide gel electrophoresis (SDS-page) and were purchased from Bio-Rad. Running buffer contained Tris/glycine with SDS. Electrophoresis was performed at constant voltage (125 V) in a Bio-Rad mini-protein II electrophoresis cell until the dye marker had reached the bottom of the gel. Electrophoretic transfer on to PVDF membrane (Millipore, Beford, Mass.) was performed with a Bio-Rad Trans-Blot cell. Transfer buffer consisted of glycine/ethanolamine and 20% methanol. Prior to transfer, the PVDF membrane was pre-wet in 100% methanol, rinsed with distilled water and immersed for 15 minutes in buffer. Transfer was performed at 20 V overnight. After electroblotting, membranes were stained with Pouceu S (Sigma) for 2 minutes, then rinsed with water and air dried.

20 Immunodetection was performed using a Bio-Rad chemiluminescent detection kit. The PVDF membrane was wet with 100% methanol, then rinsed with distilled water. The membrane was then incubated for 1 hour in blocking solution (1% blocking reagent in TBS) on a shaking incubator. The membrane was then incubated for one hour with primary antibody diluted in 0.5% blocking solution. Dilution of Pan-TGFI3 antibody was 1:2000 (1 .mu.g/.mu.l). The membrane was then washed twice in TBST for 10 minutes each, then washed twice with 0.5% blocking solution. The membrane was then incubated for 1 hour with POD-conjugated secondary antibody diluted 1:1000 in 0.5% blocking solution. The membrane was then washed four times with TBST for 15 minutes each. Excess buffer was then drained from the washed membrane, and it was placed in a staining dish and incubated for 30 minutes at room temperature with a mixture of solutions A and B (diluted 1:100 and incubated for 30 minutes at room temperature prior to addition). Approximately 125 .mu.l/cm sq. was added to the membrane container and incubated for 1 minute. The wet membrane was immediately placed into a plastic hybridization bag and the bubbles were removed. The membrane (protein side up) was placed into a film cassette against a sheet of X-ray film (X-Omat, Kodak, Rochester, N.Y.) and was exposed for 1 minute, then developed. Either no immunoreactive bands or high MW bands. (>50,000 kD) were observed in native or heat treated tears. Treatment of tears with n-acetylcysteine and heating, HCl, or HCl plus DTT resulted in immunoreactive bands at 110 kD, and 12.5 kD using TGF.beta. specific antisera (FIG. 9). These bands correspond to the published MWs of pro-TGF.beta. complexes and monomeric TGF.beta..

21 Taken together, these results indicate than native human tears contain a small amount of biologically active TGF.beta. (approximately 3.8 ng/ml), and a greater amount of latent TGF.beta. that can be released by a variety of physiochemical techniques. TGF.beta.1 is the predominant isoform in tears. Our finding of TGF.beta.1 production by human lacrimal gland secretory acini coupled with the previously reported relative lack of immunoreactivity of human ocular surface epithelia for TGF.beta. (Pasquale, L. R. et al. Invest. Ophthalmol. Vis. Sci. 1993;94:23-30) (only superficial limbal epithelia were positive) suggests that some, if not the majority, of TGF.beta. in human tears may be produced by the lacrimal gland.

CLAIMS:

What is claimed is:

1. A method of ameliorating a tear deficiency condition comprising the steps of administering to the ocular surface a pharmaceutically effective amount of an ophthalmological composition comprising a pharmaceutically effective amount of TGF.beta. in a pharmaceutically acceptable carrier.

2. The method according to claim 1 for the tear deficiency condition is dry eye.

3. The method according to claim 2 wherein said dry eye disease is Sjogren's Syndrome.

4. The method according to claim 2 wherein the dry eye condition results from a condition selected from the group consisting of hyperproliferation, squamous metaplasia, loss of goblet cells, and abnormal terminal differentiation.

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L10: Entry 27 of 29 File: JPAB Sep 2, 1997

PUB-NO: JP409227401A
DOCUMENT-IDENTIFIER: JP 09227401 A
TITLE: THERAPEUTIC AGENT FOR KERATOPATHY AND PROTECTANT FOR CORNEA

PUBN-DATE: September 2, 1997

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APPL-NO: JP08067028
APPL-DATE: February 15, 1996

INT-CL (IPC): A61 K 38/00; A61 K 9/08

ABSTRACT:

PROBLEM TO BE SOLVED: To find new effects of an apolipoprotein J in the ophthalmic region.

SOLUTION: This therapeutic agent for keratopathy comprises an apolipoprotein J as an active ingredient and is useful for keratopathy such as dry eyes, abrasion of the corneal epithelium, keratitis or corneal ulcer. The protectant for the cornea or a restoration adjuvant for the cornea comprising the apolipoprotein J as the active ingredient is obtained.

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L6: Entry 11 of 14 File: USPT Apr 3, 1990

US-PAT-NO: 4914088
DOCUMENT-IDENTIFIER: US 4914088 A

TITLE: Dry eye treatment solution and method

DATE-ISSUED: April 3, 1990

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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APPL-NO: 07/ 111874 [PALM]
DATE FILED: October 23, 1987

PARENT-CASE:
CROSS REFERENCE TO RELATED APPLICATION This application is a continuation-in-part of copending U.S. patent application Ser. No. 07/033,185 filed Apr. 2, 1987 now abandoned.

INT-CL: [04] A61K 31/66

US-CL-ISSUED: 514/76; 514/75
US-CL-CURRENT: 514/76; 514/75

FIELD-OF-SEARCH: 514/75, 514/76

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected Search ALL

	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
☐	4421748	December 1983	Trager	424/78 X
☐	4677099	June 1987	Shinitzky	514/76 X

ART-UNIT: 118

PRIMARY-EXAMINER: Dixon, Jr.; William R.

ASSISTANT-EXAMINER: Hunter, Jr.; James M.

ABSTRACT:

A method and composition for treating dry eye. The method comprises addition of a positively or negatively charged, complex phospholipid to the ocular surface of the eye. The phospholipid is desirably added in a treatment composition, preferably in the form of an aqueous emulsion. It is believed that the phospholipid component of the treatment composition permits replication of a tear film.

27 Claims, 0 Drawing figures
Exemplary Claim Number: 1

BRIEF SUMMARY:

1 BACKGROUND OF THE INVENTION

2 1. Introduction

3 This invention relates to treatment of a condition known as dry eye and more particularly, to the treatment of dry eye by topical application of certain charged phospholipids to the ocular surface.

4 2. Description of the Prior Art

5 It is known that dry eye is a condition of the eye and or the adnexa that usually causes a feeling of discomfort such as ocular dryness, grittiness, burning, soreness or scratching, dependent upon the subject and his condition. Many theories have been offered to explain the possible causes of dry eye. These theories range from the simple to the complex and include inadequate Meibomian gland secretion, insufficient tear volume, mucous deficiency, evaporative losses from the tear film and failure to form an adequate tear film. Proposed causes for dry eye, treatment and symptoms are all described in a compendium of papers on the subject edited by Holly, The Preocular Tear Film In Health, Disease, and Contact Lens Wear, The Dry Eye Institute, Lubock, Texas, 1986, incorporated herein by reference.

6 The most common treatment for dry eye involves alleviation of dry eye sumptoms by topical application of a tear substitute that adds a volume of liquid to the anterior surface of the eye and related adnexa. Typical tear substitute compositions comprise water soluble, aqueous polymer compositions. Such compositions include, for example, saline solutions of polyvinyl alcohols, hydroxypropylmethyl celluloses or carboxymethyl celluloses. U.S. Pat. No. 4,421,748 teaches an artificial tear composition comprising an aqueous hypotonic solution of lecithin and a viscosity adjusting agent such as a solution soluble cellulose.

7 Methods used to quantify the effectiveness of tear substitutes for dry eye treatment solutions have not been standardized, and many methods used in the art to quantify the results obtained with such tear-substitute compositions are often inaccurate. For this reason, it is known that reported relief of dry eye symptoms using known tear substitutes varies considerably from subject to subject, and regardless of the method used to quantify relief using a tear substitute, relief often does not exceed several minutes.

8 The symptoms associated with dry eye are often exacerbated with subjects using contact lenses. In some cases, contact lens intolerance is caused in part or in total by the condition of dry eye and the symptoms thereof. For many subjects, contact lens intolerance is not overcome by topical application of tear substitutes.

9 For the reasons given above, there is a need for improved compositions and processes for dry eye treatment. In particular, there is a need for a dry-eye-treatment composition that is easy to use, that provides a longer period of relief from dry eye symptoms, and which permits use of contact lenses by subjects having an intolerance to contact lens use due to a dry eye condition or symptoms.

10 SUMMARY OF THE INVENTION

11 The subject invention provides an improved dry eye treatment process and composition. The invention is based in part upon a means for correcting a principal deficiency in the tear film by topical application to the ocular surface of a complex phospholipid having a net positive or negative charge under conditions of use, which phospholipid, in contact with the ocular surface, is capable of replicating a tear film layer that is believed to be functionally equivalent to the tear film layer present in a healthy eye.

12 The tear film over the eye is reported to be a complex coating comprising three separate layers. The inner layer in contact with the ocular surface of the eye is reported to be primarily composed of mucous and is believed to render the hydrophobic epithelial cell surface hydrophilic. It is possible that this layer also contains other materials including phospholipid derivatives. The middle layer of the tear film is an aqueous layer. This layer is the thickest portion of the tear film and is a source of moisture for the eye and is further believed to function as an optical planarizing layer. The outer layer of the tear film, at its interface with the atmosphere and the eye, is an oily, naturally occurring lipid layer. The lipid layer is reported to act as a barrier that prevents evaporation of the aqueous layer, (Mishima and Maurice: The Oily Layer of The Tear Film and Evaporation From the Corneal Surface, Exp. Eye Res. 1961; 1: 39-45).

13 The lipid component of the tear film is believed primarily to originate from secretions of the Meibomian glands. It is formed from these secretions and is continuously replenished over the aqueous layer of the tear film during blinking due to the eyelid spreading the lipid over the surface of the eye. By constantly spreading the lipid over the eye during blinking, the tear film is maintained, and evaporation of the aqueous middle layer of the tear film is minimized.

14 A cause of dry eye is believed to result from a deficiency in the lipid layer. This deficiency may result from an inadequacy in the quantity of secretion from the Meibomian glands or an inadequacy in the quality of the secretion. Regardless of the cause of the deficiency, it is believed that the compromised layer fails to act as an adequate barrier against evaporation of the aqueous portion of the tear film thus resulting in one form of the condition known as dry eye.

15 With recognition of the most prevalent causes of dry eye, the subject invention provides a dry eye treatment process and composition for practice of the process. The treatment process comprises topical application to the ocular surface of a complex phospholipid having a net positive or negative charge under conditions of use. The phospholipid is applied to the eye in any suitable manner, and is typically applied in the form of a treatment composition such as a solution, if sufficiently soluble in its carrier liquid, a salve, ointment, suspension, or any other suitable form known to the art.

16 The preferred treatment composition is an emulsion. Upon contact of the phospolipid with the eye, it is believed that the phospholipid disperses over the ocular surface and forms a film over the eye that replicates the lipid layer that would be formed by the spreading of a naturally occurring lipid secreted from the Meibomian glands over the surface of the eye during blinking. Because the phospholipid, when applied to the eye, carries a net charge, it is believed that the aligned molecules repel each other such that complex aggregate formation is prevented and the integrity of the phospholipid film is maintained. It is believed that the film formed from the phospholipid acts as a barrier, reducing evaporation of the aqueous layer, thereby preserving the tear film.

17 Relief of dry eye symptoms by treatment in accordance with the invention is at least several fold the relief provided by prior art treatment compositions available in the marketplace. Films formed by application of the phospolipid to the eye are long lasting. Though it would be expected that a stable film formed over the eye would cause blurring, in practice, it has been found that blurring is no more severe than that blurring resulting from the application of

prior-art-treatment compositions for dry eye symptoms or even physiological saline.

18 DESCRIPTION OF THE PREFERRED EMBODIMENTS

19 As described above, dry eye treatment in accordance with the invention is accomplished by topical application to the ocular surface of a complex phospholipid having a net charge under conditions of use. Topical application is by application of a treatment composition where the phospholipid is contained in a liquid vehicle. The composition may be in the form of an emulsion, solution, salve, ointment, etc. Preferably the phospholipid is homogeneously distributed throughout the vehicle and most preferably is in the form of an aqueous emulsion. The term "homogeneous" for purposes herein means that a separate layer of the phospholipid in the composition is not visible to the naked eye, though microscopic examination of preferred compositions of the invention might reveal a laminar structure, where layers are uniformly distributed throughout the composition.

20 Phospholipids, including their plasmalogen analogs, suitable for purposes of the invention are those known in the art to be complex and known to carry a net positive or negative charge under conditions of use.

21 It is known that complex phospolipids contain a polar group at one end of their molecular structure and a non-polar group at the opposite end of their molecular structure. A discussion of phospolipids can be found in Lehninger, Biochemistry, 2 ed., Worth Publishers, New York, pp. 279-306, incorporated herein by reference.

22 Many complex phospolipids are known to the art. They differ in size, shape and the electric charge of their polar head groups. Phosphoglycerides are compounds where one primary hydroxyl group of glycerol is esterified to phosphoric acid, and the other two hydroxyl groups are esterified with fatty acids. The parent compound of the series is, therefore, the phosphoric acid ester of glycerol. This compound has an asymmetric carbon atom and, therefore, the term phosphoglycerides includes stereoisomers.

23 All phosphoglycerides have a negative charge at the phosphate group at pH 7, and the pK.sub.a of this group is in the range of 1 to 2. The head groups of phosphatidylinositol, phosphatidylglycerol including diphosphatidylglycerols (having the common name cardiolipins) and the phosphatidylsugars have no electric charge, and all are polar because of their high hydroxyl group content. Because of the negative charge of the phosphate group and the absence of a charge in the head group, the net charge of each of these materials is negative, and these materials are within the scope of the invention. Likewise, the head group of phosphatidylserine contains an alpha-amino group (pK.sub.a =10) and, a carboxyl group (pK.sub.a =3) and therefore, the molecule contains two negative charges and one positive charge at pH 7.0, giving it a net negative charge whereby this compound is also within the scope of the invention.

24 Complex phospholipids having a net positive charge are also within the scope of this invention but are lesser preferred because of the price and scarcity of these compounds. Examples of positively charged complex phospholipids within the scope of the invention are those containing the basic acyl amino acid groups. Such compounds are a sub-group within the family of the O-aminoacylphosphatidylglycerols.

25 In contrast to the charged phospholipids, the head groups of phosphatidylethanolamine and phosphatidylcholine (lecithin) have a positive charge at pH 7, and, thus, at this pH, these two phosphoglycerides are dipolar zwitterions with no net electric charge. Such compounds are not within the scope of this invention.

26 Of the phospholipids discussed above, the net-charged phosphoglycerides are preferred for purposes of the invention. A more preferred class of phosphoglycerides are represented by the following generic formula: ##STR1##

where R and R' are each fatty acid residues preferably having from 8 to 24 carbon atoms; X is hydrogen, a polyol or a 3'-O-aminoacylphosphatidylglycerol; and M is one equivalent of a countercation. R and R' are typically common natural fatty acids having an even or odd number of carbon atoms; they may be the same or may differ from each other; and they may be saturated, monounsaturated or polyunsaturated. Examples of fatty acid residues include palmitate, stearate, oleate, linoleate, octanoate, dodecate, lignocerate, etc.

27 The most preferred composition for purposes of this invention will be a mixture of complex phospholipids where each phospholipid component has a net negative charge. The most preferred phospholipids are the phosphatidylglycerols, including cardiolipins, and phosphatidylinositols.

28 Most phospholipids are water insoluble. However, for application to the eye, it is desirable that the phospholipid be homogeneously distributed throughout an aqueous medium. For those few phospholipids having a solubility within a useful concentration range for use as a treatment composition, a simple aqueous solution of the phospholipid in saline is satisfactory. For those phospholipids that are essentially water insoluble, an aqueous composition in the form of an emulsion may be used. An emulsion provides a treatment composition where the phase containing the phospholipid component is homogeneously distributed throughout the aqueous vehicle. An emulsion is readily formed by agitating one or more complex phospholipids and physiologic saline while warming the composition to a temperature in excess of the melting point of the phospholipid components. Agitation is continued at the elevated temperature until a homogeneous dispersion is obtained. Agitation is preferably mechanical agitation. Emulsification by sonification, which leads to the formation of unstable vesicles or liposomes, is undesirable. An emulsifying agent is desirably added to the formulation to stabilize the emulsion for long term storage, extended shelf life, and thermal stability. Phosphatidylcholine is a suitable emulsifying agent, though other emulsifying agents can be used if desired.

29 The concentration of the phospholipid in the treatment composition may vary within wide limits. A treatment composition containing the complex phospholipid in an amount as low as 0.01 weight percent provides some benefit. When the treatment composition is in the form of an emulsion, compositions containing the phospholipid in elevated concentrations approaching collapse of the emulsion into separate aqueous and phospholipid phases is possible. A clinically practical concentration range for the phospholipid in its vehicle varies from about 0.05 to 7.0 percent phospholipid by weight, and more preferably varies from about 0.1 and 5.0 weight percent. It should be noted that the most desired concentration for the phospholipid in the aqueous composition will vary from subject to subject.

30 Other additives may be present in the phospholipid treatment composition including neutral lipids such as one or more triglycerides, cholesterol esters, the natural waxes and cholesterol; higher molecular weight isoprenoids; stabilizers; preservatives; pH adjustors to provide a composition preferably having a pH between about 7.0 and 7.4; salt in sufficient concentration to form an isotonic composition; medicants; etc, all as would be obvious to those skilled in the art.

31 If the treatment composition is in the form of an emulsion, other additives in the treatment composition are preferably added prior to the formation of the emulsion using simple mixing techniques. The concentration of the additive is dependent upon the specific additive used and may vary between 0.00001 and 25.0 percent of the total composition.

32 For some subjects, neutral lipids comprise a desired class of additives because it is believed that they augment a second component of the lipid film located on the non-polar side of the complex phospholipid monolayer.

33 The method of application of the phospholipids of the invention to the subject is by topical application of the treatment composition to the ocular surface.

Conventional means for dispensing the treatment composition are suitable. For example, application of the treatment composition may be by spray dispenser, eyedrop, sponge, etc. The amount of the phospholipid component of the treatment composition is small. If the treatment composition is in the form of an aqueous solution or emulsion, a single drop from a conventional eye dropper is satisfactory. Preferred dosage comprises application of one drop several times daily. The preferred dosage is believed to be capable of providing relief for periods of time at least several fold compared to commercial formulations, dependent upon the subject, as will be illustrated in the examples that follow.

34 Though not wishing to be bound by theory, it is believed that upon contact of the phospholipid treatment composition with the eye, a molecularly aligned monolayer complex phospholipid film forms over the aqueous layer of the tear film where the molecular alignment is augmented by the charge on the phospholipid. It is also possible that a portion of the phospholipid may be found in other portions of the tear film, such as in the first layer of the tear film is contact with the epithelial cell surface.

35 The invention will be better understood by reference to the examples that follow. In the examples, the efficacy of the treatment solution was determined using three test procedures described as follows:

36 EVALUATION OF BREAK UP TIME (BUT)

37 The normal tear film maintains an uninterrupted surface for a finite but adequate period of time to both protect the eye and to permit clear vision without blinking. Blinking restores and maintains the tear film. Certain conditions, such as forcibly restraining blinking, can cause the tear film to become discontinuous. The measurement of the time (in seconds) required for the tear film to evidence areas of discontinuity when blinking is suspended, is known as break up time (BUT). In the prior art, BUT has been measured by adding fluorescein, a fluorescent dye, and observing the stained tear film with light passed through a cobalt blue filter. However, the use of the dye in BUT evaluation presents another variable and was avoided for the tests reported herein. Instead, optical imaging was employed using a method described by Norn M. S.: "Tear Film Break Up Time. A Review". in Holly F, The Preocular Tear Film, 1986, pp. 52-54. The test is performed with an ophthalmometer as proposed by Norn without the use of the fluorescein dye. For each subject, a baseline BUT value was established. Larger BUTs represent a more stable tear film. The effectiveness of tear substitutes was investigated by comparison of BUT for various test solutions and controls versus a baseline BUT value for each eye tested. To evaluate BUT for a given test solution, the test solution is added to the eye by depressing the lower eyelid and placing one standard drop of the test solution into the inferior fornix (the space between the lower eyelid and the eye). The eyelid is then released, the subject is asked to blink at five second intervals, and one minute is allowed for the tear film to stabilize. The BUT measurement is then made as described by Norn with the ophthalmometer.

38 To obtain baseline data, the BUT test is performed prior to the addition of a test solution and then following the use of a test solution. Where multiple test solutions were used on a single subject on a given day, a recovery (waiting) time is required between trials. The recovery procedure involves irrigation (flushing) of the front surface of the eyes with sterile saline. Following irrigation, a recovery time of 20 minutes is provided to allow for stabilization of the tear film. If BUT is not consistent with initial baseline findings, further recovery time is provided until BUT times have returned to normal. In the examples, all BUT results are given in seconds.

39 MEASUREMENT OF VISUAL BLUR TIME (VBT)

40 Any liquid added to the tear film would be expected to cause a blur until the tear film returns to a form providing optimal optical characteristics. The test described below is intended to quantify blur time as a consequence of addition of a test solution.

41 To qualify a subject prior to the addition of a test solution, the subject is directed to a standard vision testing chart to determine if the subject's habitual tear film results in a visual blur with a measurable VBT. A subject qualifies if the habitual tear film does not produce a measurable VBT i.e., in the absence of a test solution, VBT is expected to be 0.

42 The test solution is added to the eye by depressing the lower eyelid and placing one standard drop into the inferior fornix (the space between the lower lid and eye). The eyelid is released, the subject's attention is directed to a standard vision testing chart, and the subject is asked to blink at 5 second intervals until the blur resolves. The time required for the blur to resolve, in seconds, is recorded as the VBT. This time may vary from several seconds to minutes. Times of less than 30 seconds are desirable. In the examples that follow, all times are in seconds.

43 EVALUATION OF LIPID THICKNESS (LT)

44 Quantification of the thickness of the lipid layer utilized methods reported by Guillon, J. P., "Tear Film Structure and Contact Lenses," in Holly F., The Preocular Tear Film in Health, Disease and Contact Lens Wear, Chapt. 85, pp. 914-939. This method allows approximation of the thickness of the lipid layer by observation of colored interference patterns over a representative portion of the corneal surface as set forth in the chart that follows:

Color	Lipid Tickness (nm)
None	< 90
Yellow	90
Yellow to Brown	90-<170
Blue	170
Intense Blue	>170

45 It should be recognized that subjectivity is involved in the reporting of observations of color. By necessity, color observation is subjective. Moreover, with this test, there are variations in the color at different locations on the corneal surface. Further, a lipid layer reflecting a minimal or fleeting blue coloration was considered to have a thickness of 170 nm. A lipid layer reflecting a more intense blue coloration, or a blue coloration over a greater portion of the test surface of the eye, was interpreted as having a thickness greater than 170 nm.

46 An effort was made to select subjects exhibiting dry eye symptoms with ocular findings indicating a deficient lipid layer and dysfunctional Meibomian glands. Again, baseline data was obtained for each eye prior to the addition of a test solution. Prior to the addition of any test solution, lipid thickness of a tear film was observed over a period of time generally ranging from 2 to 5 minutes. Observations were also made after 5, 15 and 60 minutes to determine if findings were consistent. For all tests and for each subject, the number of trials performed varied from 3 to 6, and an average of the results obtained are reported in the examples. If consistent findings were demonstrated, the subject qualified for study.

47 LT evaluation was made following instillation of one drop of treatment solution followed by observation until the blur produced by the solution had resolved. Observation times were standardized at 1 minute, 5 minutes, 15 minutes, 60 minutes and when possible, 180 minutes. Subjects presenting tear film conditions without detectable difference between eyes were selected, and one eye was evaluated with a control solution and the other with the test solution. Tests were of double blind design. Minor changes to the procedure described above are

given in the examples.

DETAILED DESCRIPTION:

1 EXAMPLE 1

2 This example sets forth a generalized procedure for the make-up of phospholipid test solutions and identifies test solutions used in the examples that follow.

3 A test phospholipid in a desired concentration was added to one liter of a borate buffered (pH 7.2), sorbic acid stabilized physiological saline (0.9% NaCl in sterile deionized water) with stirring. While stirring, the mixture was heated to a temperature immediately above the melting point of the test phospholipid to assist in its distribution in the saline. The mixture was maintained at elevated temperature with stirring for a time sufficient for the formation of a thickened and stable emulsion. This time typically varied from 20 to 60 minutes. The emulsion was then cooled to 24.degree. C.

4 Test solutions prepared in accordance with the above procedure, controls used, and codes assigned to test solutions follow:

CPC	A mixed phospholipid emulsion containing 7.0% of a product sold by Fisher Chemical as "Lecithin" and having a composition determined by analysis as follows: Lysophosphatidic acid 0.59% Phosphatidylglycerol 1.00% Lysophosphatidylethanolamine 0.74% Phosphatidic acid 7.08% Cardiolipin 2.14% Phosphatidylethanolamine 14.49% Sphinogomeylin 1.00% Phosphatidylinositol 9.58% Phosphatidylcholine 14.64% Neutral lipids balance
0.7 CPC	CPC diluted 10 fold
CPCO	CPC diluted with 50% of a neutral lipid oil
0.7 CPCO	0.7 CPC diluted with 50% of a neutral lipid oil
PG-7	0.7% emulsion of phosphatidylinositol
PG-8	0.7% emulsion of phosphatidylglycerol
PG-10	0.7% solution of pure phosphatidylcholine (lecithin)
PG-11	0.7% solution of pure phosphatidylcholine (lecithin) diluted by 50% with neutral lipid
PG-15	0.7% solution of pure cardiolipin
PGS	Same as CPC but a second batch
10PGS	PGS diluted 10 fold
10PGSO	10 PGS diluted with 50% of a neutral lipid oil
HTC	Control solution comprising commercial artiicial tear product available from Cooper Vision Pharmaceuticals Inc. believed to be a solution of water soluble polymer

	identified as HypoTears .RTM. Dry Eye Solution.
UNC	Control solution comprising commercial saline product available from Cooper Vision Pharmaceuticals Inc. and identified as Unisol .RTM. saline solution.
TGC	Control solution analyzed as an aqueous mixture of phospholipids consisting essentially of a major portion of phosphatidylcholine and a minor portion of lysophosphatidylinositol and lyso-phosphatidylcholine, and a hydroxyethyl cellulose in a hypotonic vehicle sold under the trademark TearGard .RTM. by Bio Products Inc.
SESC	Sorbic acid preserved saline solution sold under the trademark Sensitive Eyes .RTM. by Bausch and Lomb Inc.

5 PG-7, PG-8, PG-15 and mixtures of these materials in equal concentration constitute the most preferred embodiment of the invention.

6 EXAMPLE 2

7 A subject was selected and Break-Up-Time, (BUT), Visual Blur Time (VBT) and Lipid Thickness (LT) were determined prior to adding a test solution to obtain baseline data with the following results obtained:

	Baseline Data Left Eye	Right Eye
BUT (sec.)	8	11
VBT (sec.)	0	0
LT (nm)		
1 min.	<90	<90
5 mins.	<90	<90
15 mins.	<90	<90
60 mins.	<90	<90
180 mins.	<90	<90

8 Based upon the baseline data, the two eyes were considered equal for BUT, VBT and LT tests. The tests were then repeated on contralateral eyes to evaluate the HTC and 0.7 CPC test solutions. The HTC solution was added to the right eye and the 0.7 CPC solution added to the left eye. The results obtained follow:

	Test Data Left Eye (0.7 CPC)	Right Eye (HTC)
BUT (sec.)	33	14
VBT (sec.)	13	15
LT (nm)		

1 min.	.gtoreq.170	<90
5 mins.	.gtoreq.170	<90
15 mins.	.gtoreq.170	<90
60 mins.	.gtoreq.170	<90
180 mins.	90-170	<90

9 EXAMPLE 3

10 The procedures of Example 2 were repeated using 11 additional subjects. Again, HTC was added to the right eye (R) and 0.7 CPC was added to the left eye (L). The results obtained are reported in the following table:

Subject	Eye	BUT (sec.) Baseline	BUT (sec.) Test	VBT (sec.) Baseline	VBT (sec.) Test	LT (in nm) Baseline	1 Min.	5 Min.	15 Min.	60 Min.	180 Min.
3-1	R	12	11	0	12	<90	90	90	<90	<90	<90
	L	14	32	0	12	<90	>170	170	>170	>170	170
3-2	R	11	27	0	16	<90	90	<90	<90	<90	<90
	L	9	41	0	12	<90	>170	>170	>170	>170	170
3-3	R	6	13	0	11	<90	<90	<90	<90	<90	<90
	L	4	43	0	11	<90	>170	>170	170	170	90 < 170
3-4	R	8	23	0	16	<90	<90	<90	<90	<90	<90
	L	6	39	0	11	<90	>170	170	170	170	90
3-5	R	9	11	0	13	<90	90	90	90	<90	<90
	L	7	32	0	10	<90	>170	170	170	170	90
3-6	R	11	5	0	22	<90	90	<90	<90	<90	<90
	L	12	27	0	18	<90	>170	>170	90	90	90
3-7	R	10	8	0	15	<90	<90	<90	<90	<90	<90
	L	9	14	0	14	<90	>170	>170	170	90	90
3-8	R	16	11	0	15	<90	90	90	90	<90	<90
	L	14	39	0	14	<90	>170	170	170	90	90
3-9	R	15	11	0	11	<90	<90	<90	<90	<90	<90
	L	13	32	0	9	<90	170	170	90 < 170		
										90 < 170	
											90 < 170
3-10	R	9	8	0	12	<90	90	90	<170	<170	<170
	L	8	31	0	11	<90	>170	>170	>170	170	90 < 170
3-11	R	14	12	0	9	<90	<90	< 90	<90	<90	<90
	L	12	20	0	12	<90	>170	170	90 < 170		
										90 < 170	
											90 < 170

11 In the above tests, an improvement in BUT was observed using 0.7 CPC. The data indicates that the tear film is more stable after the addition of 0.7 CPC than prior to the use of the composition and more stable than that achieved with HTC.

The VBT is essentially the same as for the HTC commercial control product establishing that blurring is not exacerbated with the use of 0.7 CPC use. The LT shows improvement both following instillation of 0.7 CPC and following from 1 to 3 hours after instillation. The HTC control does not produce such improvement.

12 EXAMPLE 4

13 This example measures blur caused by the compositions of the invention at two different concentration levels with and without addition of a neutral lipid (oil). The example compares 4 compositions prepared in accordance with the invention with a commercial formulation for dry eye treatment. Further subjects were used for test purposes with results set forth in the following table:

Visual Blur Time (sec.) Patient						
	Baseline	HTC	0.7 CPC	CPC	0.7 CPCO	CPCO
4-1	0	16	7	115	15	72
4-2	0	11	18	75	29	125
4-3	0	26	20	105	19	210
4-4	0	16	8	260	8	215
4-5	0	12	12	133	9	60
4-6	0	12	16	103	25	95
4-7	0	7	11	145	13	125
4-8	0	9	10	145	6	125
4-9	0	21	14	125	16	185
4-10	0	10	13	195	9	165
4-11	0	16	18	140	23	165
4-12	0	22	23	150	26	60

14 EXAMPLE 5

15 Another subject was tested to obtain baseline values for BUT, VBT and LT. The procedures of Example 2 were used. Following qualification for both the right and left eyes, test solutions of Example 1 were used and BUT, VBT and LT were determined for this subject following the installation of the test solution. The results are set forth in the following table:

BUT (sec.)		VBT (sec.)		LT (in nm)					
Solution	Baseline	Test	Baseline	Test	Baseline	1 Min.	5 Min.	15 Min.	60 Min.	180 Min.
HTC	9	17	0	18	<90	90	90	<90	<90	<90
UNC	9	12	0	12	<90	90	90	<90	<90	<90

PG-7 9	38 0	12 <90	>170 >170	>170 >170 170					
PG-8 9	34 0	14 <90	>170 >170	>170 170	90 < 170				
PG-10									
9	16 0	11 <90	90 < 170						
			90	<90	<90	<90			
PG-11									
9	19 0	12 <90	90 < 170						
			90	<90	<90	<90			
PG-15									
9	29 0	13 <90	>170 >170	>170 >170	90 < 170				
10 PGS									
9	30 0	10 <90	90 < 170						
			>170	90 < 170					
				90 < 170					
				90 < 170					

16 EXAMPLE 6

17 The procedure of Example 5 was repeated with six additional subjects. The results are given below:

Test	BUT (sec.)		VBT (sec.)		LT (in nm)				
Solution									
Subject									
Baseline									
Test									
Baseline									
Test									
Baseline									
1 Min.									
5 Min	15 Min.								
60 Min.									
180 Min.									
HTC									
1	5	6 0	47	90	90	90	90	90	90
2	8	9 0	30	90	90	90	90	90	90
3	13	11 0	20	90	>170	90	90	90	90
4	8	6 0	12	90	90	90	90	90	90
5	16	20 0	5	90	>170	90	90	90	90
6	10	7 0	9	<90	90	90	<90	<90	<90
Avg.	10	10 0	20						
UNC									
1	5	5 0	14	90	90	90	90	90	90
2	8	7 0	20	90	90	90	90	90	90
3	13	6 0	10	90	<90	90	90	90	90
4	8	9 0	10	90	90	90	90	90	90
5	16	17 0	4	90	>170	90	90	90	90
6	10	10 0	7	<90	90	<90	<90	<90	<90
Avg.	10	9 0	11						
PG-7									
1	5	20 0	11	90	>170	>170	>170	>170	90-170
2	8	23 0	13	90	>170	>170	>170	>170	90< 170
3	13	31 0	14	90	>170	>170	>170	>170	>170
4	8	33 0	11	90	>170	>170	>170	90 < 170	
									90 < 170
5	16	39 0	5	90	>170	>170	>170	>170	90 < 170

```
      6     10     26 0     9    90    >170   >170   >170   90 < 170
                                                             90 < 170
      Avg.  10     29 0     11
PG-8
      1     5      15 0     13   90    >170   >170   >170   >170   90 < 170
      2     8      21 0     29   90    >170   >170   >170   >170   90 < 170
      3     13     37 0     39   90    >170   >170   >170   >170   170
      4     8      21 0     10   90    >170   >170   >170   90 < 170
                                                             90 < 170
      5     16     51 0     5    90    >170   >170   >170   >170   90 < 170
      6     10     29 0     3    90    >170   >170   >170   90 < 170
                                                             90 < 170
      Avg.  10     29 0     17
PG-10
      1     5      7  0     16   90    90 < 170
                                        90 < 170
                                              90     90     90
      2     8      10 0     11   90    90 < 170
                                        90 < 170
                                              90     90     90
      3     13     12 0     15   90    >170   90 < 170
                                              90     90     90
      4     8      10 0     7    90    >170   90     90     90     90
      5     16     15 0     4    90    >170   >170   90     90     90
      6     10     11 0     6    <90   90     90     <90    <90    <90
      Avg.  10     11 0     10
PG-11
      1     5      6  0     16   90    90 <  170
                                        90 < 170
                                              90     90     90
      2     8      9  0     17   90    90 < 170
                                        90 < 170
                                              90     90     90
      3     13     13 0     10   90    >170   90     90     90     90
      4     8      9  0     18   90    >170   90     90     90     90
      5     16     16 0     5    <90   90 < 170
                                        90     90     90     90
      6     10     13 0     11   <90   90     90     <90    <90    <90
      Avg.  10     11 0     13
PG-15
      1     5      23 0     12   90    >170   >170   >170   90 < 170
                                                             90 < 170
      2     8      17 0     21   90    >170   >170   >170   90 < 170
                                                             90 < 170
      3     13     27 0     23   90    >170   >170   >170   >170   90 < 170
4           8      31 0     10   Not Done
5           16     46 0     6    Not Done
      6     10     31 0     7    <90   >170   >170    >170 90-170
                                                             90 < 170
      Avg.  10     29 0     13
10 PGS
      1     5      16 0     12   90    >170   >170   >170   90 < 170
                                                             90 < 170
      2     8      14 0     8    90    >170   >170   >170   90 < 170
                                                             90 < 170
      3     13     23 0     13   90    >170   >170   90 < 170
                                                       90 < 170
                                                             90
      4     8      23 0     9    90    >170   >170   90 < 170
                                                       90 < 170
                                                             90
      5     16     31 0     4    90    >170   >170   90 < 170
                                                       90 < 170
                                                             90
      6     10     21 0     16   <90   >170   >170   >170   90     90
```

Avg. 10 21 0 9

18 Of particular interest in the above examples are the results obtained using the test solution identified as PG-10. PG-10 is a solution of pure phosphatidylcholine, a neutral phospholipid known commercially as lecithin. The results using this phospholipid are inferior to the results obtained using a charged phospholipid in accordance with the invention.

19 EXAMPLE 7

20 An additional subject was selected and baseline BUT data collected. The right eye BUT was 9 seconds and the left, 10 seconds. Both eyes were considered equal. VBT was found to be 0. LT was found to be less than 90 nm.

21 BUT, VBT and LT were evaluated for test solutions identified in Example 1 using the procedures of Example 2. The results follow:

Solution	BUT (sec.) Baseline	BUT (sec.) Test	VBT (sec.) Baseline	LT (in nm) Baseline	LT (in nm) 1 Min.	LT (in nm) 5 Min.	LT (in nm) 15 Min.	LT (in nm) 60 Min.	LT (in nm) 180 Min.
HTC	10	14 0	17	<90	90	90	<90	<90	<90
UNC	10	12 0	13	<90	90	90	<90	<90	<90
PG-7	10	31 0	11	<90	>170	>170	>170	>170	170
PG-8	10	30 0	13	<90	>170	>170	>170	170	90 < 170
TGC	10	13 0	175	<90	90	90	<90	<90	<90

22 EXAMPLE 8

23 The procedure of Example 7 was expanded to an additional five subjects with results as follow:

Test Solution	Subject	BUT (sec.) Baseline	BUT (sec.) Test	VBT (sec.) Baseline	LT (in nm) Test	LT (in nm) Baseline	LT (in nm) 1 Min.	LT (in nm) 5 Min.	LT (in nm) 15 Min.

									60 Min.	180 Min.	
HTC											
	1	7	13	0	18	<90	90	90	<90	<90	<90
	2	9	11	0	22	<90	90	90	<90	<90	<90
	3	14	13	0	6	90	90 < 170				
								90 < 170			
									90	90	90
	4	11	10	0	9	90	90 < 170				
								90 < 170			
									90	90	90
	5	9	13	0	13	<90	90	90	90	<90	<90
	Avg.	10	12	0	10						
UNC											
	1	7	10	0	14	<90	90	90	<90	<90	<90
	2	9	12	0	10	<90	90	90	<90	<90	<90
	3	14	11	0	8	90	90 < 170				
								90 < 170			
									90	90	90
	4	11	13	0	15	90	90 < 170				
								90 < 170			
									90	90	90
	5	9	11	0	9	<90	90	90	<90	<90	<90
	Avg.	10	11	0	13						
PG-7											
	1	7	37	0	13	<90	>170	>170	>170	>170	90 < 170
	2	9	27	0	10	<90	>170	>170	>170	>170	90 < 170
	3	14	29	0	9	90	>170	>170	>170	170	90 < 170
	4	11	32	0	10	90	>170	>170	>170	170	90 < 170
	5	9	36	0	8	<90	>170	>170	>170	170	90 < 170
	Avg.	10	32	0	10						
PG-8											
	1	7	32	0	11	<90	>170	>170	>170	170	90 < 170
	2	9	23	0	8	<90	>170	>170	>170	>170	170
	3	14	31	0	7	90	>170	>170	>170	170	170
	4	11	26	0	12	90	>170	>170	>170	170	90 < 170
	5	9	28	0	9	<90	>170	>170	>170	170	90 < 170
	Avg	10	28	0	10						
TGC.sup.2											
	1	7	12	0	240	<90	90	90	<90	<90	<90
	2	9	9	0	180	<90	90	90	<90	<90	<90
	3	14	11	0	60	90	90 < 170				
								90 < 170			
									90	90	90
	4	11	10	0	180	90	90 < 170				
								90 < 170			
									90	90	90
	5	9	12	0	240	<90	90	90	<90	<90	<90
	Avg.	10	11	0	180						

24 EXAMPLE 9

25 Twenty subjects were selected that were both users and non users of contact lenses. Each was provided with treatment solution in 5 cc bottles blind coded for objectivity. The codes of Example 1 were used. The materials tested were as follows:

26 Coded Material

27 PGS

28 10 PGS

29 10 PGSO

30 CON

31 HTC

32 In these examples, sorbic acid preserved saline solution carrier was selected for preparation of the emulsions identified as 10 PGS, PGS and 10 PGSO in distinction to the compositions described in Example 1.

33 The solutions were evaluated for comfort and improvement of classic dry eye symptoms. Preference for lack of blur and other undesirable phenomena were noted. The following contralateral test plan was implemented:

Day	Right Eye	Left Eye
1-4	CON	10 PGSO
5-8	10 PGSO	CON
9-12	10 PGS	PGS
13-16	PGS	10 PGS

34 The results follow:

Composition	Preference No. of Subjects
CON	0
10 PGS	13
PGS	1
10 PGSO	4
No choice	2
Total	20

35 The majority of the subjects preferred the 10-PGS, but 4 (20% of population) preferred 10-PGSO (with the addition of oil).

36 EXAMPLE 10

37 The procedure of Example 9 was repeated with 6 additional subjects with solutions coded as follows:

38 Coded Material

39 10 PGS

40 PG-7

41 PG-10

42 SESC

43 TGC

44 The following contralateral test plan was used:

Day	Right Eye	Left Eye
1-4	HTC	SESC
5-8	SESC	HTC
9-12	TGC	10 PGS
13-16	10 PGS	TGC
17-20	PG-7	PG-10
21-24	PG-10	PG-7

45 The results follow:

Composition	Preference No. of Subjects
HTC	0
SESC	0
TGC	0
10PGS	1
PG-7	5
PG-10	0
No choice	0
Total	6

46 EXAMPLE 11

47 The procedure of Example 9 was repeated with six additional subjects with solutions coded as follows:

48 Coded Material

49 HTC

50 TGC

51 SESC

52 10 PGS

53 The following contralateral test plan was implemented.

Day	Right Eye	Left Eye
1-4	HTC	SESC
5-8	SESC	HTC
9-12	TGC	10 PGS
13-16	10 PGS	TGC

54 The results were as follows:

Composition	Preference No. of Subjects
HTC	0
SESC	0
TGC	0
10 PGS	6
No choice	0
Total	6

55 There are many forms of eye disease which are the result or which are associated with dry eye conditions. The phospholipid treatment compositions of the invention provide relief for these conditions. Evaluation of these compositions with subjects evidencing forms of superior limbal conjunctivitis, traumatized bulbar and palpebral conjunctiva, and related lid disease have shown significant relief from associated pain and also improvement in the tissue associated with these diseases. This is considered significant for the medical treatment of anterior segment surface disease.

CLAIMS:

We claim:

1. An artificial tear film over the surface of an eye comprising a first layer in direct contact with the ocular surface, an aqueous layer over the first layer, and a layer of a complex phospholipid having a net positive or negative charge over the aqueous layer.

2. The tear film of claim 1 where the phospholipid layer has polar groups extending towards the aqueous layer and non-polar groups extending away from the aqueous layer.

3. The tear film of claim 1 where the phospholipid layer is selected from the group of phosphatidylglycerols, phosphatidylinositols, diphosphatidylglycerols, phosphatidylsugars and mixtures thereof.

4. The tear film of claim 1 where the phospholipid layer is of a phospholipid conforming to the following general formula: ##STR2## where R and R' are each fatty acid residues, X is hydrogen, a polyol or an 3'-O-aminoacylphosphatidylglycerol, and M is a cation.

5. The tear film of claim 4 where R and R' are the same.

6. The tear film of claim 1 where the phospholipid is phosphatidylglycerol.

7. The tear film of claim 1 where the phospholipid is phosphatidylinositol.

8. The tear film of claim 1 where the phospholipid is a cardiolipin.

9. A stable aqueous emulsion eye treatment composition having a pH of from about 7.0 to 7.4 containing one or more complex phospholipids having a net positive or negative charge under conditions of use in an amount of at least 0.01 percent by weight of the total weight of the treatment composition.

10. The treatment composition of claim 9 where the phospholipid composition contains the phospholipid in an amount of from 0.05 to 7.0 percent by weight.

11. The treatment composition of claim 9 containing a neutral lipid in addition to the phospholipid.

12. The treatment composition of claim 9 where the phospholipid is selected from the group of phosphatidylglycerols, phosphatidylinositols, diphosphatidylglycerols, phosphatidylsugars and mixtures thereof.

13. The treatment composition of claim 9 where the phospholipid conforms to the following general formula: ##STR3## where R and R' are each fatty acid residues, X is hydrogen, a polyol or a 3'-O-aminoacylphosphatidylglycerol, and M is a cation.

14. The treatment composition of claim 13 where R and R' are the same.

15. The treatment composition of claim 9 where the phospholipid is phosphatidylglycerol.

16. The treatment composition of claim 9 where the phospholipid is phosphatidylinositol.

17. The treatment composition of claim 9 where the phospholipid is a cardiolipin.

18. A method for treating the eye comprising the formation of an artificial tear film over the eye by the topical application to the ocular surface of a treatment composition that contains one or more complex phospholipids having a net negative or positive charge under conditions of use in an amount of at least 0.01 percent by weight of the total weight of the treatment composition.

19. The dry eye treatment method of claim 18 where the aqueous composition is in the form of a stable aqueous emulsion.

20. The dry eye treatment method of claim 19 where the phospholipid composition contains the phospholipid in an amount of from 0.5 to 7.0 percent by weight.

21. The dye eye treatment method of claim 19 where the phospholipid is selected from the group of phosphatidylglycerol, phosphatidylinositol and cardiolipins.

22. The dry eye treatment method of claim 19 where the phospholipid conforms to the following general formula: ##STR4## where R and R' are each fatty acid residues, X is hydrogen, a polyol or a 3'-O-aminoacylphosphatidylglycerol, and M is a cation.

23. The dry eye treatment method of claim 22 where R and R' are the same.

24. The dry eye treatment method of claim 22 where R and R' differ from each other.

25. The dry eye traatment method of claim 19 where the phospholipid is phosphatidylglycerol.

26. The dry eye treatment method of claim 19 where the phospholipid is phosphatidylinositol.

27. The dry eye treatment method of claim 19 where the phospholipid is a cardiolipin.

WEST

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L3: Entry 11 of 11 File: USPT Jul 29, 1997

US-PAT-NO: 5652274
DOCUMENT-IDENTIFIER: US 5652274 A

TITLE: Therapeutic-wound healing compositions and methods for preparing and using same

DATE-ISSUED: July 29, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Martin; Alain	Ringoes	NJ	08551	

APPL-NO: 08/ 445813 [PALM]
DATE FILED: May 22, 1995

PARENT-CASE:
This is a continuation-in-part of U.S. application Ser. No. 08/187,435 filed Jan. 27, 1994; now abandoned, which was a continuation of U.S. application Ser. No. 07/798,392 filed Nov. 26, 1991; now abandoned, which was a continuation-in-part of U.S. application Ser. No. 07/663,500 filed Mar. 1, 1991, now abandoned.

INT-CL: [06] A61 K 31/45, A61 K 31/07, A61 K 31/34, A61 K 47/00

US-CL-ISSUED: 514/724; 514/461, 514/562, 514/567, 514/725, 514/774, 514/784
US-CL-CURRENT: 514/724; 514/461, 514/562, 514/567, 514/725, 514/774, 514/784

FIELD-OF-SEARCH: 514/724, 514/725, 514/461, 514/774, 514/784, 514/562, 514/567

PRIOR-ART-DISCLOSED:

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Search Selected | Search ALL

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ART-UNIT: 125

PRIMARY-EXAMINER: Criares; Theodore J.

ABSTRACT:

This invention pertains to therapeutic wound healing compositions for protecting and resuscitating mammalian cells. In one embodiment, the therapeutic wound healing composition comprises (a) pymvate, (b) an antioxidant, and (c) a mixture of saturated and unsaturated fatty acids. In another embodiment, the therapeutic wound healing composition comprises (a) pyruvate, (b) lactate, and (c) a mixture of saturated and unsaturated fatty acids. In yet another embodiment, the therapeutic wound healing composition comprises (a) an antioxidant and (b) a mixture of saturated and unsaturated fatty adds. In still yet another embodiment, the therapeutic wound healing composition comprises (a) lactate, (b) an antioxidant, and (c) a mixture of saturated and unsaturated fatty acids. This invention also pertains to wound healing compositions combined with a medicament which is useful for treating injured mammalian cells to form augmented wound healing compositions such as immunostimulating-wound healing compositions, antiviral-wound healing compositions, antikeratolytic-wound healing compositions, anti-inflammatory-wound healing compositions, antifungal-wound healing compositions, acne treating-wound healing compositions, sunscreen-wound healing compositions, dermatological-wound healing compositions, antihistamine-wound healing compositions, antibacterial-wound healing compositions, and bioadhesive-wound healing compositions. This invention also pertains to wound healing compositions combined with a cytotoxic agent to form cytoprotective-wound healing compositions useful for protecting and reducing injury to mammalian cells and to razor cartridges comprising the wound healing compositions. This invention also pertains to methods for preparing and using the wound healing compositions and the topical and ingestible pharmaceutical products in which the therapeutic compositions may be used.

16 Claims, 90 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 77

DETAILED DESCRIPTION:

1 DETAILED DESCRIPTION OF THE INVENTION

2 Applicant has discovered therapeutic wound healing compositions for preventing and reducing injury to mammalian cells and increasing the resuscitation rate of injured mammalian cells. Cells treated with the therapeutic wound healing compositions of the present invention show decreased levels of hydrogen peroxide production, increased resistance to cytotoxic agents, increased rates of proliferation, and increased viability. Cellular cultures containing the therapeutic wound healing compositions showed enhanced differentiation and

proliferation over control cultures and rapidly formed attachments or tight junctions between the cells to form an epidermal sheet. Wounded mammals treated with the therapeutic wound healing compositions show significantly improved wound closing and healing over untreated mammals and mammals treated with conventional healing compositions. The wound healing compositions may be used alone or in combination with other medicaments.

3 In Embodiment One (I), the therapeutic wound healing compositions are used alone. In a first aspect of Embodiment One (I.A), the therapeutic wound healing composition comprises (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof, (b) an antioxidant, and (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells. In a second aspect of Embodiment One (I.B), the therapeutic wound healing composition comprises (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyrovic acid, and mixtures thereof, (b) lactate selected from the group consisting of lactic acid, pharmaceutically acceptable salts of lactic acid, and mixtures thereof, and (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells. In a third aspect of Embodiment One (I.C), the therapeutic wound healing composition comprises (a) an antioxidant and (b) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells. In a fourth aspect of Embodiment One (I.D), the therapeutic wound healing composition comprises (a) lactate selected from the group consisting of lactic acid, pharmaceutically acceptable salts of lactic acid, and mixtures thereof, (b) an antioxidant, and (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells.

4 In Embodiment Two (II), the therapeutic wound healing compositions of Embodiment One (I.A-D) are combined with a medicament (M) which is useful for treating injured mammalian cells to form augmented wound healing compositions (I.A-D+M).

5 In a first aspect of Embodiment Two (I.A-D+M1), the invention pertains to a therapeutic immunostimulating-wound healing composition which comprises a therapeutically effective amount of an immunestimulating agent (M1) and a wound healing composition of the present invention (I.A-D). Immunostimulating agents can stimulate the immune system in a patient to kill an infecting organism but do not promote the wound healing process. Wound healing compositions can increase the resuscitation rate of injured mammalian cells and the proliferation rate of new mammalian cells to replace dead cells but do not kill infecting organisms. The combination of an immunestimulating agent and a wound healing composition results in an immunostimulating-wound healing composition which acts synergistically to enhance wound repair in both the upper and lower portions of the skin. The therapeutic immunostimulating-wound healing compositions are superior in stimulating both the extent of re-epithelialization as well as the extent of tissue integrity and leukocyte infiltration in the dermis.

6 In a second aspect of Embodiment Two (I.A-D+M2), the invention pertains to a therapeutic antiviral-wound healing composition which comprises a therapeutically effective mount of an antiviral agent (M2) and a wound healing composition of the present invention (I.A-D). Antiviral agents can reduce virus titers in a patient but do not promote the wound healing process. Wound healing compositions can increase the resuscitation rate of injured mammalian cells and the proliferation rate of new mammalian cells to replace dead cells but do not reduce virus titers. The combination of an antiviral agent and a wound healing composition results in a therapeutic antiviral-wound healing composition which reduces the size, duration, and severity of oral and vaginal wounds suffered from viruses such as herpes.

7 In a third aspect of Embodiment Two (I.A-D+M3), the invention pertains to a therapeutic antikeratolytic-wound healing composition which comprises a

therapeutically effective amount of an antikeratolytic agent (M3) and a wound healing composition of the present invention (I.A-D). Antikeratolytic agents can reduce scaling and dryness in a patient but do not promote the wound healing process. Wound healing compositions can increase the resuscitation rate of injured mammalian cells and the proliferation rate of new mammalian cells to replace dead cells but do not reduce the proliferation of cells. The combination of an antikeratolytic agent and a wound healing composition results in a therapeutic antikeratolytic-wound healing composition which reduces the duration and severity of psoriasis.

8 In a fourth aspect of Embodiment Two (I.A-D+M4), the invention pertains to a therapeutic anti-inflammatory-wound healing composition which comprises a therapeutically effective amount of an anti-inflammatory agent (M4) and a wound healing composition of the present invention (I.A-D). Anti-inflammatory agents can reduce inflammation in a patient but do not promote the wound healing process. Wound healing compositions can increase the resuscitation rate of injured mammalian cells and the proliferation rate of new mammalian cells to replace dead cells but do not reduce inflammation. The combination of an anti-inflammatory agent and a wound healing composition results in a therapeutic anti-inflammatory-wound healing composition which reduces the duration and severity of intimation.

9 In a fifth aspect of Embodiment Two (I.A-D+M5), the invention pertains to a therapeutic antifungal-wound healing composition which comprises a therapeutically effective amount of a first antifungal agent (M5) selected from the group consisting of lactic acid and sorbic acid and a wound healing composition of the present invention (I.A-D). Antifungal agents can treat fungal infections in a patient but do not promote the wound healing process. Wound healing compositions can increase the resuscitation rate of injured mammalian cells and the proliferation rate of new mammalian cells to replace dead cells but do not treat fungal infections. The combination of an antifungal agent and a wound healing composition results in a therapeutic antifungal-wound healing composition which reduces the duration and severity of fungal infections. The therapeutic antifungal-wound healing compositions may further comprise a therapeutically effective amount of a second antifungal agent and an anti-inflammatory agent.

10 In a sixth aspect of Embodiment Two (I.A-D+M6), the invention pertains to a therapeutic acne treating-wound healing composition for the topical treatment of acne vulgaris which comprises a therapeutically effective amount of tretinoin (M6) and a wound healing composition of the present invention (I.A-D). Tretinoin is useful for the topical treatment of acne vulgaris but is known to induce excessive redness, edematous blistering or crusting, and severe local erythema and peeling at the site of application. Wound healing compositions can increase the resuscitation rate of injured mammalian cells and the proliferation rate of new mammalian cells to replace dead cells. The combination of tretinoin and a wound healing composition results in a therapeutic acne treating-wound healing composition which reduces the duration and severity of acne vulgaris and the irritation associated with tretinoin. This invention also relates to methods for employing the therapeutic acne treating-wound healing compositions to treat wrinkles.

11 In a seventh aspect of Embodiment Two (I.A-D+M7), the invention pertains to a therapeutic sunscreen-wound healing composition useful to minimize and treat sunburn damage which comprises a therapeutically effective amount of a sunscreen agent and an anti-inflammatory agent (sunscreen agent and anti-inflammatory agent collectively referred to as M7) and a wound healing composition of the present invention (I.A-D). Sunscreen agents can help prevent sunburn by screening ultra violet light but do not heal injured mammalian cells. Anti-inflammatory agents can reduce inflammation (erythema) in a patient but do not promote the wound healing process. Wound healing compositions can increase the resuscitation rate of injured mammalian cells and the proliferation rate of new mammalian cells to replace dead cells. Wound healing compositions can also minimize oxygen radical damage from ultra violet light. The combination of a sunscreen agent, an anti-inflammatory agent, and a wound healing composition

results in a therapeutic sunscreen-wound healing compositions useful for minimizing and treating sunburn damage. The sunscreen-wound healing compositions may optionally contain a therapeutically effective amount of a topical anesthetic to further reduce the severity of sunburn.

12 In an eighth aspect of Embodiment Two (I.A-D+M8), the invention pertains to a therapeutic dermatological-wound healing composition useful to minimize and treat diaper dermatitis which comprises a therapeutically effective amount of a buffering agent and an anti-inflammatory agent (buffering agent and anti-inflammatory agent collectively referred to as M8) and a wound healing composition of the present invention (I.A-D). Buffering agents can help prevent diaper dermatitis by neutralizing ammonia but do not heal injured mammalian cells. Anti-inflammatory agents can reduce inflammation (erythema) in a patient but do not promote the wound healing process. Wound healing compositions can increase the resuscitation rate of injured mammalian cells and the proliferation rate of new mammalian cells to replace dead cells. The combination of a buffering agent, an anti-inflammatory agent, and a wound healing composition results in a therapeutic dermatological-wound healing compositions useful for minimizing and treating diaper dermatitis. The dermatological-wound healing compositions may optionally contain a therapeutically effective amount of a topical antiseptic to further reduce the duration and severity of diaper dermatitis.

13 In a ninth aspect of Embodiment Two (I.A-D+M9), the invention pertains to a therapeutic antihistamine-wound healing composition which comprises a therapeutically effective amount of a topical antihistamine agent (M9) and a wound healing composition of the present invention (I.A-D). Topical antihistamines are useful for the topical treatment of skin irritations but are potentially sensitizing. Wound healing compositions can increase the resuscitation rate of injured mammalian cells and the proliferation rate of new mammalian cells to replace dead cells. The combination of a topical antihistamine and a wound healing composition results in a therapeutic antihistamine-wound healing composition which reduces the duration and severity of itching associated with skin irritations and the sensitizing effect associated with topical antihistamines.

14 In a tenth aspect of Embodiment Two (I.A-D+M10), the invention pertains to a therapeutic antibacterial-wound healing composition which comprises a therapeutically effective amount of an antibacterial agent (M10) and a wound healing composition of the present invention (I.A-D). Antibacterial agents are useful for treating bacterial infections but do not promote the wound healing process. Wound healing compositions can increase the resuscitation rate of injured mammalian cells and the proliferation rate of new mammalian cells to replace dead cells. The combination of an antibacterial agent and a wound healing composition results in a therapeutic antibacterial-wound healing composition which reduces the size, duration, and severity of infected wounds.

15 In an eleventh aspect of Embodiment Two (I.A-D+M11), the invention pertains to a therapeutic bioadhesive-wound healing composition which comprises a bioadhesive agent (M11) and a therapeutically effective amount of a wound healing composition of the present invention (I.A-D). The bioadhesive agent comprises a water-swellable but water-insoluble fibrous cross-linked material which adheres to live or freshly killed mucous membranes or skin tissues. The combination of a bioadhesive agent and a wound healing composition results in a therapeutic bioadhesive-wound healing composition which can increase the resuscitation rate of injured mammalian cells and the proliferation rate of new mammalian cells to replace dead cells and thereby reduce the duration and severity of wounds. The therapeutic bioadhesive-wound healing compositions may further comprise medicaments such as immunostimulating agents, antiviral agents, antikeratolytic agents, anti-inflammatory agents, antifungal agents, tretinoin, sunscreen agents, dermatological agents, topical antihistamine agents, antibacterial agents, cytotoxic agents, and the like.

16 In Embodiment Three (III), the therapeutic wound healing compositions of Embodiment One (I.A-D) are combined with a cytotoxic agent (X) to form

cytoprotective-wound healing compositions (I.A-D+X) useful for preventing and reducing injury to mammalian cells from a medicament having cytotoxic properties. Cells treated with the cytoprotective compositions of the present invention show decreased levels of hydrogen peroxide production, increased resistance to cytotoxic agents, increased rates of proliferation, and increased viability. The wound healing compositions may be administered to cells concurrently with a cytotoxic agent or the wound healing compositions may be administered to cells prior to administration of a cytotoxic anticancer agent to selectively protect non-cancerous cells in the presence of cancerous cells. Because cancerous cells have a rapid metabolism, cancerous cells will rapidly consume the protective wound healing composition and will not be protected by the wound healing compositions when the chemotherapeutic medicament is subsequently administered.

17 In another aspect of Embodiment Three (III), the cytoprotective-wound healing composition (I.E+X) comprises a cytotoxic agent (X) and a wound healing composition (I.E) comprising: (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof; and (b) an antioxidant.

18 In Embodiment Four (IV), the therapeutic wound healing compositions of Embodiment One (I.A-D) are affixed to a razor cartridge (R) to form disposable razor cartridges comprising wound healing compositions (I.A-D+R). The integral wound healing composition delivery system is preferably in the form of a solid strip of a water-soluble encapsulating agent comprising the wound healing composition premixed with a polymeric delivery system. Wound healing compositions can increase the resuscitation rate of injured mammalian cells and the proliferation rate of new mammalian cells to replace dead cells. Applicants have found that binding a wound healing composition to a razor cartridge results in a therapeutic razor cartridge which can reduce the duration and severity of shaving cuts and nicks.

19 In another aspect of Embodiment Four (IV), the disposable razor cartridges (IV.F+R) comprise a wound healing composition comprising an antioxidant (F) affixed to a razor cartridge (R).

20 The term "injured cell" as used herein means a cell that has any activity disrupted for any reason. For example, an injured cell may be a cell that has injured membranes or damaged DNA, RNA, and ribosomes, for example, a cell which has (a) injured membranes so that transport through the membranes is diminished resulting in an increase in toxins and normal cellular wastes inside the cell and a decrease in nutrients and other components necessary for cellular repair inside the cell, (b) an increase in concentration of oxygen radicals inside the cell because of the decreased ability of the cell to produce antioxidants and enzymes, or (c) damaged DNA, RNA, and ribosomes which must be repaired or replaced before normal cellular functions can be resumed. The term "resuscitation" of injured mammalian cells as used herein means the reversal of cytotoxicity, the stabilization of the cellular membrane, an increase in the proliferation rate of the cell, and/or the normalization of cellular functions such as the secretion of growth factors, hormones, and the like. The term "cytotoxicity" as used herein means a condition caused by a cytotoxic agent that injures the cell. Injured cells do not proliferate because injured cells expend all energy on cellular repair. Aiding cellular repair promotes cellular proliferation.

21 The term "prodrug", as used herein, refers to compounds which undergo biotransformation prior to exhibiting their pharmacological effects. The chemical modification of drugs to overcome pharmaceutical problems has also been termed "drug latentiation." Drug latentiation is the chemical modification of a biologically active compound to form a new compound which upon in vivo enzymatic attack will liberate the parent compound. The chemical alterations of the parent compound are such that the change in physicochemical properties will affect the absorption, distribution and enzymatic metabolism. The definition of drug latentiation has also been extended to include nonenzymatic regeneration of the parent compound. Regeneration takes place as a consequence of hydrolytic,

dissociative, and other reactions not necessarily enzyme mediated. The terms prodrugs, latentiated drugs, and bioreversible derivatives are used interchangeably. By inference, latentiation implies a time lag element or time component involved in regenerating the bioactive parent molecule in vivo. The term prodrug is general in that it includes latentiated drug derivatives as well as those substances which are converted after administration to the actual substance which combines with receptors. The term prodrug is a generic term for agents which undergo biotransformation prior to exhibiting their pharmacological actions. In the case where the administered drug is not the active agent, but rather is biotransformed to the active agent, the term "prodrug" also includes compounds which may not necessarily undergo biotransformation to the administered drug but may undergo biotransformation to the active agent which exhibits the desired pharmacological effect.

22 The term "metabolite", as used herein, refers to any substance produced by metabolism or by a metabolic process. "Metabolism", as used herein, refers to the various chemical reactions involved in the transformation of molecules or chemical compounds occurring in tissue and the cells therein.

23 I. Wound Healing Compositions

24 A. Embodiment One (I.A-D)

25 The cells which may be treated with the therapeutic wound healing compositions in the present invention are mammalian cells. Although applicant will describe the present therapeutic wound healing compositions as useful for treating mammalian epidermal keratinocytes and mammalian monocytes, applicant contemplates that the therapeutic wound healing compositions may be used to protect or resuscitate all mammalian cells. Keratinocytes are representative of normal mammalian cells and are the fastest proliferating cells in the body. The correlation between the reaction of keratinocytes to injury and therapy and that of mammalian cells in general is very high. Monocytes are representative of specialized mammalian cells such as the white blood cells in the immune system and the organ cells in liver, kidney, heart, and brain. The mammalian cells may be treated in vivo and in vitro.

26 Epidermal keratinocytes are the specialized epithelial cells of the epidermis which synthesize keratin, a scleroprotein which is the principal constituent of epidermis, hair, nails, horny tissue, and the organic matrix of the enamel of teeth. Mammalian epidermal keratinocytes constitute about 95% of the epidermal cells and together with melanocytes form the binary system of the epidermis. In its various successive stages, epidermal keratinocytes are also known as basal cells, prickle cells, and granular cells.

27 Monocytes are mononuclear phagocytic leukocytes which undergo respiratory bursting and are involved in reactive oxygen mediated damage within the epidermis. Leukocytes are white blood cells or corpuscles which may be classified into two main groups: granular leukocytes (granulocytes) which are leukocytes with abundant granules in the cytoplasm and nongranular leukocytes (nongranulocytes) which are leukocytes without specific granules in the cytoplasm and which include the lymphocytes and monocytes. Phagocyte cells are cells which ingest microorganisms or other cells and foreign particles. Monocytes are also known as large mononuclear leukocytes, and hyaline or transitional leukocytes.

28 Epidermal keratinocytic cells and monocytic cells have multiple oxygen generating mechanisms and the degree to which each type of mechanism functions differs in each type of cell. In monocytes, for example, the respiratory bursting process is more pronounced than in epidermal keratinocytes. Hence, the components in the therapeutic wound healing compositions of the present invention may vary depending upon the types of cells involved in the condition being treated.

29 As set out above, in a first aspect of Embodiment One (I.A), the therapeutic wound healing composition for treating mammalian cells, preferably epidermal

keratinocytes, comprises (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyrovic acid, and mixtures thereof, (b) an antioxidant, and (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells. In a second aspect of Embodiment One (I.B), the therapeutic wound healing composition for treating mammalian cells, preferably epidermal keratinocytes, comprises (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyrovic acid, and mixtures thereof, (b) lactate selected from the group consisting of lactic acid, pharmaceutically acceptable salts of lactic acid, and mixtures thereof, and (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells. In a third aspect of Embodiment One (I.C), the therapeutic wound healing composition for treating mammalian cells, preferably epidermal keratinocytes, comprises (a) an antioxidant and (b) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells. In a fourth aspect of Embodiment One (I.D), the therapeutic wound healing composition for treating mammalian cells, preferably monocytes, comprises (a) lactate selected from the group consisting of lactic acid, pharmaceutically acceptable salts of lactic acid, and mixtures thereof, (b) an antioxidant, and (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells.

30 Pyruvic acid (2-oxopropanoic acid, .alpha.-ketopropionic acid, CH.sub.3 COCOOH) or pyruvate is a fundamental intermediate in protein and carbohydrate metabolism and in the citric acid cycle. The citric acid cycle (tricarboxylic acid cycle, Kreb's cycle) is the major reaction sequence which executes the reduction of oxygen to generate adenosine triphosphate (ATP) by oxidizing organic compounds in respiring tissues to provide electrons to the transport system. Acetyl coenzyrne A ("active acetyl") is oxidized in this process and is thereafter utilized in a variety of biological processes and is a precursor in the biosynthesis of many fatty acids and sterols. The two major sources of acetyl coenzyme A are derived from the metabolism of glucose and fatty acids. Glycolysis consists of a series of transformations wherein each glucose molecule is transformed in the cellular cytoplasm into two molecules of pyruvic acid. Pyruvic acid may then enter the mitochondria where it is oxidized by coenzyme A in the presence of enzymes and cofactors to acetyl coenzyme A. Acetyl coenzyme A can then enter the citric acid cycle.

31 In muscle, pyrovic acid (derived from glycogen) can be reduced to lactic acid during anerobic metabolism which can occur during exercise. Lactic acid is reoxidized and partially retransformed to glycogen during rest. Pyruvate can also act as an antioxidant to neutralize oxygen radicals in the cell and can be used in the multifunction oxidase system to reverse cytotoxicity.

32 The pyruvate in the present invention may be selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyrovic acid, prodrugs of pyruvic acid, and mixtures thereof. In general, the pharmaceutically acceptable salts of pyruvic acid may be alkali salts and alkaline earth salts. Preferably, the pyruvate is selected from the group consisting of pyrovic acid, lithium pyruvate, sodium pyruvate, potassium pyruvate, magnesium pyruvate, calcium pyruvate, zinc pyruvate, manganese pyruvate, methyl pyruvate, .alpha.-ketoglutaric acid, and mixtures thereof. More preferably, the pyruvate is selected from the group of salts consisting of sodium pyruvate, potassium pyruvate, magnesium pyruvate, calcium pyruvate, zinc pyruvate, manganese pyruvate, and the like, and mixtures thereof. Most preferably, the pyruvate is sodium pyruvate.

33 The amount of pyruvate present in the therapeutic wound healing compositions of the present invention is a therapeutically effective amount. A therapeutically effective amount of pyruvate is that amount of pyruvate necessary for the inventive composition to prevent and reduce injury to mammalian cells or

increase the resuscitation rate of injured mammalian cells. The exact amount of pyruvate is a matter of preference subject to such factors as the type of condition being treated as well as the other ingredients in the composition. In a preferred embodiment, pyruvate is present in the therapeutic wound healing composition in an amount from about 10% to about 50%, preferably from about 20% to about 45%, and more preferably from about 25% to about 40%, by weight of the therapeutic wound healing composition.

34 L-Lactic acid ((S)-2-hydroxypropanoic acid, (+) .alpha.-hydroxypropionic acid, CH.sub.3 CHOHCOOH) or lactate occurs in small quantities in the blood and muscle fluid of mammals. Lactic acid concentration increases in muscle and blood after vigorous activity. Lactate is a component in the cellular feedback mechanism and inhibits the natural respiratory bursting process of cells thereby suppressing the production of oxygen radicals.

35 The lactate in the present invention may be selected from the group consisting of lactic acid, pharmaceutically acceptable salts of lactic acid, prodrugs of lactic acid, and mixtures thereof. In general, the pharmaceutically acceptable salts of lactic acid may be alkali salts and alkaline earth salts. Preferably, the lactate is selected from the group consisting of lactic acid, lithium lactate, sodium lactate, potassium lactate, magnesium lactate, calcium lactate, zinc lactate, manganese lactate, and the like, and mixtures thereof. More preferably, the lactate is selected from the group consisting of lactic acid, sodium lactate, potassium lactate, magnesium lactate, calcium lactate, zinc lactate, manganese lactate, and mixtures thereof. Most preferably, the lactate is lactic acid.

36 The amount of lactate present in the therapeutic wound healing compositions of the present invention is a therapeutically effective amount. A therapeutically effective amount of lactate is that amount of lactate necessary for the inventive composition to prevent and reduce injury to mammalian cells or increase the resuscitation rate of injured mammalian cells. For an ingestible composition, a therapeutically effective amount of lactate is that amount necessary to suppress the respiratory bursting process of white blood cells to protect and resuscitate the mammalian cells. In general, a therapeutically effective amount of lactate in an ingestible composition is from about 5 to about 10 times the amount of lactate normally found in serum. The exact amount of lactate is a matter of preference subject to such factors as the type of condition being treated as well as the other ingredients in the composition. In a preferred embodiment, lactate is present in the therapeutic wound healing composition in an amount from about 10% to about 50%, preferably from about 20% to about 45%, and more preferably from about 25% to about 40%, by weight of the therapeutic wound healing composition.

37 Antioxidants are substances which inhibit oxidation or suppress reactions promoted by oxygen or peroxides. Antioxidants, especially lipid-soluble antioxidants, can be absorbed into the cellular membrane to neutralize oxygen radicals and thereby protect the membrane. The antioxidants useful in the present invention may be selected from the group consisting of all forms of Vitamin A including retinal and 3,4-didehydroretinal, all forms of carotene such as Alpha-carotene, .beta.-carotene (beta, .beta.-carotene), gamma-carotene, delta-carotene, all forms of Vitamin C (D-ascorbic acid, L-aseorbic acid), all forms of tocopherol such as Vitamin E (Alpha-tocopherol, 3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltri-decyl)-2H-1-benzopy ran-6-ol), .beta.-tocopherol, gamma-tocopherol, delta-tocopherol, tocoquinone, tocotrienol, and Vitamin E esters which readily undergo hydrolysis to Vitamin E such as Vitamin E acetate and Vitamin E succinate, and pharmaceutically acceptable Vitamin E salts such as Vitamin E phosphate, prodrugs of Vitamin A, carotene, Vitamin C, and Vitamin E, pharmaceutically acceptable salts of Vitamin A, carotene, Vitamin C, and Vitamin E, and the like, and mixtures thereof. Preferably, the antioxidant is selected from the group of lipid-soluble antioxidants consisting of Vitamin A, .beta.-carotene, Vitamin E, Vitamin E acetate, and mixtures thereof. More preferably, the antioxidant is Vitamin E or Vitamin E acetate. Most preferably, the antioxidant is Vitamin E acetate.

38 The amount of antioxidant present in the therapeutic wound healing compositions of the present invention is a therapeutically effective amount. A therapeutically effective amount of antioxidant is that amount of antioxidant necessary for the inventive composition to prevent and reduce injury to mammalian cells or increase the resuscitation rate of injured mammalian cells. The exact amount of antioxidant is a matter of preference subject to such factors as the type of condition being treated as well as the other ingredients in the composition. In a preferred embodiment, the antioxidant is present in the therapeutic wound healing composition in an amount from about 0.1% to about 40%, preferably from about 0.2% to about 30%, and more preferably from about 0.5% to about 20%, by weight of the therapeutic wound healing composition.

39 The mixture of saturated and unsaturated fatty acids in the present invention are those fatty acids required for the repair of mammalian cellular membranes and the production of new cells. Fatty acids are carboxylic acid compounds found in animal and vegetable fat and oil. Fatty acids are classified as lipids and are composed of chains of alkyl groups containing from 4 to 22 carbon atoms and 0-3 double bonds and characterized by a terminal carboxyl group, --COOH. Fatty acids may be saturated or unsaturated and may be solid, semisolid, or liquid. The most common saturated fatty acids are butyric acid (C.sub.4), lauric acid (C.sub.12), palmitic acid (C.sub.16), and stearic acid (C.sub.18). Unsaturated fatty acids are usually derived from vegetables and consist of alkyl chains containing from 16 to 22 carbon atoms and 0-3 double bonds with the characteristic terminal carboxyl group. The most common unsaturated fatty acids are oleic acid, linoleic acid, and linolenic acid (all C.sub.18 acids).

40 In general, the mixture of saturated and unsaturated fatty acids required for the repair of mammalian cellular membranes in the present invention may be derived from animal and vegetable fats and waxes, prodrugs of saturated and unsaturated fatty acids useful in the present invention, and mixtures thereof. For example, the fatty acids in the therapeutic wound healing composition may be in the form of mono-, di-, or trigylcerides, or free fatty acids, or mixtures thereof, which are readily available for the repair of injured cells. Cells produce the chemical components and the energy required for cellular viability and store excess energy in the form of fat. Fat is adipose tissue stored between organs of the body to furnish a reserve supply of energy. The preferred animal fats and waxes have a fatty acid composition similar to that of human fat and the fat contained in human breast milk. The preferred animal fats and waxes may be selected from the group consisting of human fat, chicken fat, cow fat (defined herein as a bovine domestic animal regardless of sex or age), sheep fat, horse fat, pig fat, and whale fat. The more preferred animal fats and waxes may be selected from the group consisting of human fat and chicken fat. The most preferred animal fat is human fat. Mixtures of other fats and waxes, such as vegetable waxes (especially sunflower oil), marine oils (especially shark liver oil), and synthetic waxes and oils, which have a fatty acid composition similar to that of animal fats and waxes, and preferably to that of human fats and waxes, may also be employed.

41 In a preferred embodiment, the mixture of saturated and unsaturated fatty acids has a composition similar to that of human fat and comprises the following fatty acids: butyric acid, caproic acid, caprylic acid, captic acid, laurie acid, myristic acid, myristoleic acid, palmitic acid, palmitoleic acid, stearic, oleic acid, linoleic acid, linolenic acid, arachidic acid, and gadoleic acid. Preferably, butyric acid, caproic acid, caprylic acid, capric acid, laurie acid, myristic acid, myristoleic acid, palmitic acid, palmitoleic acid, stearic, oleic acid, linoleic acid, linolenic acid, arachidic acid, and gadoleic acid are present in the mixture in about the following percentages by weight, respectively (carbon chain number and number of unsaturations are shown parenthetically, respectively): 0.2%-0.4% (C.sub.4), 0.1% (C.sub.6), 0.3%-0.8% (C.sub.8), 2.2%-3.5% (C.sub.10), 0.9%-5.5% (C.sub.12), 2.8%-8.5% (C.sub.14), 0.1%-0.6% (C.sub.14:1), 23.2%-24.6% (C.sub.16), 1.8-%3.0% (C.sub.16:1), 6.9%-9.9% (C.sub.18), 36.0%-36.5% (C.sub.18:1), 20%-20.6% (C.sub.18:2), 7.5-7.8% (C.sub.18:3), 1.1%-4.9% (C.sub.20), and 3.3%-6.4% (C.sub.20:1).

42 In another preferred embodiment, the mixture of saturated and unsaturated fatty

acids is typically chicken fat comprising the following fatty acids: lauric acid, myristic acid, myristoleic acid, pentadecanoic acid, palmitic acid, palmitoleic acid, margaric acid, margaroleic acid, stearic, oleic acid, linoleic acid, linolenic acid, arachidic acid, and gadoleic acid. Preferably, lauric acid, myristic acid, myristoleic acid, pentadecanoic acid, palmitic acid, palmitoleic acid, margaric acid, margaroleic acid, stearic, oleic acid, linoleic acid, linolenic acid, arachidic acid, and gadoleic acid are present in the mixture in about the following percentages by weight, respectively: 0.1% (C.sub.12), 0.8% (C.sub.14), 0.2% (C.sub.14:1), 0.1% (C.sub.15), 25.3% (C.sub.16), 7.2% (C.sub.16:1), 0.1% (C.sub.17), 0.1% (C.sub.17:1), 6.5% (C.sub.18), 37.7% (C.sub.18:1), 20.6% (C.sub.18:2), 0.8% (C.sub.18:3), 0.2% (C.sub.20), and 0.3% (C.sub.20:1), all percentages .+-.10%.

43 In another preferred embodiment, the mixture of saturated and unsaturated fatty acids comprises lecithin. Lecithin (phosphatidylcholine) is a phosphatide found in all living organisms (plants and animals) and is a significant constituent of nervous tissue and brain substance. Lecithin is a mixture of the diglycerides of stearic, palmitic, and oleic acids, linked to the oholine ester of phosphoric acid. The product of commerce is predominantly soybean lecithin obtained as a by-product in the manufacturing of soybean oil. Soybean lecithin contains palmitic acid 11.7%, stearic 4.0%, palmitoleic 8.6%, oleic 9.8%, linoleic 55.0%, linolenic 4.0%, C.sub.20 to C.sub.22 acids (includes arachidonic) 5.5%. Lecithin may be represented by the formula: ##STR1## wherein R is selected from the group consisting of stearic, palmitic, and oleic acid.

44 The above fatty acids and percentages thereof present in the fatty acid mixture are given as an example. The exact type of fatty acid present in the fatty acid mixture and the exact amount of fatty acid employed in the fatty acid mixture may be varied in order to obtain the result desired in the final product and such variations are now within the capabilities of those skilled in the art without the need for undue experimentation.

45 The amount of fatty acids present in the therapeutic wound healing compositions of the present invention is a therapeutically effective amount. A therapeutically effective amount of fatty acids is that amount of fatty acids necessary for the inventive composition to prevent and reduce injury to mammalian cells or increase the resuscitation rate of injured mammalian cells. The exact amount of fatty acids employed is subject to such factors as the type and distribution of fatty acids employed in the mixture, the type of condition being treated, and the other ingredients in the composition. In a preferred embodiment, the fatty acids are present in the therapeutic wound healing composition in an amount from about 10% to about 50%, preferably from about 20% to about 45%, and more preferably from about 25% to about 40%, by weight of the therapeutic wound healing composition.

46 In accord with the present invention, the therapeutic wound healing compositions of Embodiment One (I.A-D) for treating mammalian cells may be selected from the group consisting of:

47 (I.A)

48 (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

49 (b) an antioxidant; and

50 (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells;

51 (I.B)

52 (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) lactate selected from the group consisting of lactic acid, pharmaceutically acceptable salts of lactic acid, and mixtures thereof; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells;

(I.C)

(a) an antioxidant; and

(b) a mixture of sainted and unsaturated fatty acids wherein the fatty adds are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells;

(I.D)

(a) lactate selected from the group consisting of lactic add, pharmaceutically acceptable salts of lactic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty adds are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells.

Preferably, the wound healing compositions of Embodiment One (I) for treating mammalian cells, preferably epidermal keratinocytes, may be selected from the group consisting of:

(I.A)

(a) pyruvate selected from the group consisting of pyrovic acid, pharmaceutically acceptable salts of pyrovic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of sainted and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells;

(I.B)

(a) pyruvate selected from the group consisting ofpyruvic acid, pharmaceutically acceptable salts of pyrovic acid, and mixtures thereof;

(b) lactate selected from the group consisting of lactic acid, pharmaceutically acceptable salts of lactic acid, and mixtures thereof; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells; and

(I.C)

(a) an antioxidant; and

(b) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells.

More preferably, the wound healing compositions of Embodiment One (I) for treating mammalian cells, preferably epidermal keratinocytes, may be selected from the group consisting of:

75 (I.A)

76 (a) pyruvate selected from the group consisting ofpyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

77 (b) an antioxidant; and

78 (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells; and

79 (I.C)

80 (a) an antioxidant; and

81 (b) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells.

82 More preferably, the wound healing compositions of Embodiment One (I) for treating mammalian cells, preferably epidermal keratinocytes, may be selected from the group consisting of:

83 (I.A)

84 (a) pyruvate selected from the group consisting ofpyruvic acid, pharmaceutically acceptable salts of pyrovic acid, and mixtures thereof;

85 (b) an antioxidant; and

86 (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells; and

87 (I.B)

88 (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyrovic acid, and mixtures thereof;

89 (b) lactate selected from the group consisting of lactic acid, pharmaceutically acceptable salts of lactic acid, and mixtures thereof; and

90 (c) a mixture of sainted and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells.

91 Most preferably, the wound healing compositions of Embodiment One (I) for treating mammalian cells, preferably epidermal keratinocytes, comprise:

92 (I.A)

93 (a) pyruvate selected from the group consisting ofpyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

94 (b) an antioxidant; and

95 (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells.

96 Most preferably, the wound healing compositions of Embodiment One (I) for treating mammalian cells, preferably monocytes, comprise:

97 (I.D)

98 (a) lactate selected from the group consisting of lactic acid, pharmaceutically acceptable salts of lactic acid, and mixtures thereof;

99 (b) an antioxidant; and

100 (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells.

101 Throughout this disclosure, applicant will suggest various theories or mechanisms by which applicant believes the components in the therapeutic wound healing compositions and the antiviral agent function together in an unexpected synergistic manner to prevent and reduce injury to mammalian cells, increase the resuscitation rate of injured mammalian cells, and reduce viral titers. While applicant may offer various mechanisms to explain the present invention, applicant does not wish to be bound by theory. These theories are suggested to better understand the present invention but are not intended to limit the effective scope of the claims.

102 In the first aspect of Embodiment One (I.A), applicant believes that pyruvate can be transported inside a cell where it can act as an antioxidant to neutralize oxygen radicals in the cell. Pyruvate can also be used inside the cell in the citric acid cycle to provide energy to increase cellular viability, and as a precursor in the synthesis of important biomolecules to promote cellular proliferation. In addition, pyruvate can be used in the multifunction oxidase system to reverse cytotoxicity. Antioxidants, especially lipid-soluble antioxidants, can be absorbed into the cell membrane to neutralize oxygen radicals and thereby protect the membrane. The saturated and unsaturated fatty acids in the present invention are those fatty acids required for the resuscitation of mammalian cells and are readily available for the repair of injured cells and the proliferation of new cells. Cells injured by oxygen radicals need to produce unsaturated fatty acids to repair cellular membranes. However, the production of unsaturated fatty acids by cells requires oxygen. Thus, the injured cell needs high levels of oxygen to produce unsaturated fatty acids and at the same time needs to reduce the level of oxygen within the cell to reduce oxidative injury. By providing the cell with the unsaturated fatty acids needed for repair, the need of the cell for unsaturated fatty acids is reduced and the need for high oxygen levels is also reduced.

103 The combination of pyruvate inside the cell and an antioxidant in the cellular membrane functions in an unexpected synergistic manner to reduce hydrogen peroxide production in the cell to levels lower than can be achieved by use of either type of component alone. The presence of mixtures of saturated and unsaturated fatty acids in the therapeutic wound healing composition significantly enhances the ability of pyruvate and the antioxidant to inhibit reactive oxygen production. By stabilizing the cellular membrane, unsaturated fatty acids also improve membrane function and enhance pyruvate transport into the cell. Hence, the three components in the therapeutic wound healing composition of the first aspect of Embodiment One (I.A) function together in an unexpected synergistic manner to prevent and reduce injury to mammalian cells and increase the resuscitation rate of injured mammalian cells.

104 In the second aspect of Embodiment One (I.B), lactate is employed instead of an antioxidant. Antioxidants react with, and neutralize, oxygen radicals after the radicals are already formed. Lactate, on the other hand, is a component in the cellular feedback mechanism and inhibits the respiratory bursting process to suppress the production of active oxygen species. The combination of pyruvate to neutralize active oxygen species and lactate to suppress the respiratory bursting process functions in a synergistic manner to reduce hydrogen peroxide production in the cell to levels lower than can be achieved by use of either type of component alone. The presence of mixtures of saturated and unsaturated fatty acids in the therapeutic wound healing composition significantly enhances the ability ofpyruvate and lactate to inhibit reactive oxygen production. Hence, the three components in the therapeutic wound healing composition in the second aspect of Embodiment One (I.B) function together in a synergistic manner to

protect and resuscitate mammalian cells.

105 In the third aspect of Embodiment One (I.C), the presence of mixtures of saturated and unsaturated fatty acids in the therapeutic wound healing composition in this embodiment significantly enhances the ability of the antioxidant to inhibit reactive oxygen production. The combination of an antioxidant to neutralize active oxygen species and fatty acids to rebuild cellular membranes and reduce the need of the cell for oxygen functions in a synergistic manner to reduce hydrogen peroxide production in the cell to levels lower than can be achieved by either type of component alone. Hence, the components in the therapeutic wound healing composition in the third aspect of Embodiment One (I.C) function together in a synergistic manner to protect and resuscitate mammalian cells.

106 In the fourth aspect of Embodiment One (I.D), lactate is employed because the respiratory bursting process is more pronounced in monocytes than in epidermal keratinocytes. The combination of lactate to suppress the respiratory bursting process and an antioxidant to neutralize active oxygen species functions in a synergistic manner to reduce hydrogen peroxide production in the cell to levels lower than can be achieved by either component alone. The presence of mixtures of saturated and unsaturated fatty acids in the therapeutic wound healing composition in this embodiment significantly enhances the ability of lactate and the antioxidant to inhibit reactive oxygen production. Hence, the three components in the therapeutic wound healing composition in the fourth aspect of Embodiment One (I.D) function together in an unexpected synergistic manner to protect and resuscitate mammalian cells.

107 Accordingly, the combination of ingredients set out in the above embodiments functions together in an enhanced manner to prevent and reduce injury to mammalian cells and increase the resuscitation rate of injured mammalian cells. The therapeutic effect of the combination of the components in each of the above embodiments is markedly greater than that expected by the mere addition of the individual therapeutic components. Hence, applicant's therapeutic wound healing compositions for treating mammalian cells have the ability to decrease intracellular levels of hydrogen peroxide production, increase cellular resistance to cytotoxic agents, increase rates of cellular proliferation, and increase cellular viability.

108 B. Methods For Making The Therapeutic Wound Healing Compositions Of Embodiment One (I.A-D)

109 The present invention extends to methods for making the therapeutic wound healing compositions of Embodiment One (I.A-D). In general, a therapeutic wound healing composition is made by forming an admixture of the components of the composition. In a first aspect of Embodiment One (I.A), a therapeutic wound healing composition is made by forming an admixture of (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyrovic acid, and mixtures thereof, (b) an antioxidant, and (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells. In a second aspect of Embodiment One (I.B), a therapeutic wound healing composition is made by forming an admixture of (a) pyruvate selected from the group consisting of pyrovic acid, pharmaceutically acceptable salts of pyrovic acid, and mixtures thereof, Co) lactate selected from the group consisting of lactic acid, pharmaceutically acceptable salts of lactic acid, and mixtures thereof, and (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells. In a third aspect of Embodiment One (I.C), a therapeutic wound healing composition is made by forming an admixture of (a) an antioxidant and (b) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells. In a fourth aspect of Embodiment One (I.D), a therapeutic wound healing composition is made by forming an admixture of (a) lactate selected from the group consisting of lactic acid, pharmaceutically acceptable salts of lactic acid, and

mixtures thereof, (b) an antioxidant, and (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells.

110 For some applications, the admixture may be formed in a solvent such as water, and a surfactant may be added if required. If necessary, the pH of the solvent is adjusted to a range from about 3.5 to about 8.0, and preferably from about 4.5 to about 7.5, and more preferably about 6.0 to about 7.4. The admixture is then sterile filtered. Other ingredients may also be incorporated into the therapeutic wound healing composition as dictated by the nature of the desired composition as well known by those having ordinary skill in the art. The ultimate therapeutic wound healing compositions are readily prepared using methods generally known in the pharmaceutical arts.

111 In a preferred embodiment, the invention is directed to a method for preparing a therapeutic wound healing composition (I.A) for preventing and reducing injury to mammalian cells, and increasing the resuscitation rate of injured mammalian cells, which comprises the steps of admixing the following ingredients:

112 (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyrovic acid, and mixtures thereof;

113 (b) an antioxidant; and

114 (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the resuscitation of injured mammalian cells.

115 C. Methods For Employing The Therapeutic Wound Healing Compositions Of Embodiment One (I.A-D)

116 The present invention extends to methods for employing the therapeutic wound healing compositions of Embodiment One (I) in vivo and in vitro. In general, a therapeutic wound healing composition is employed by contacting the therapeutic composition with mammalian cells.

117 In a first aspect of Embodiment One (I.A), the invention is directed to a method for preventing and reducing injury to mammalian cells, and increasing the resuscitation rate of injured mammalian cells, which comprises the steps of (A) providing a therapeutic wound healing composition which comprises (a) pyruvate selected from the group consisting of pyrovic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof, (b) an antioxidant, and (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the resuscitation of injured mammalian cells, and (B) contacting the therapeutic wound healing composition with the mammalian cells.

118 In a second aspect of Embodiment One (I.B), the invention is directed to a method for preventing and reducing injury to mammalian cells, and increasing the resuscitation rate of injured mammalian cells, which comprises the steps of (A) providing a therapeutic wound healing composition which comprises (a) pyruvate selected from the group consisting of pyrovic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof, (b) lactate selected from the group consisting of lactic acid, pharmaceutically acceptable salts of lactic acid, and mixtures thereof, and (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the resuscitation of injured mammalian cells, and (B) contacting the therapeutic wound healing composition with the mammalian cells.

119 In a third aspect of Embodiment One (I.C), the invention is directed to a method for preventing and reducing injury to mammalian cells, and increasing the resuscitation rate of injured mammalian cells, which comprises the steps of (A) providing a therapeutic wound healing composition which comprises (a) an antioxidant, and (b) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the resuscitation of injured mammalian cells, and (B) contacting the therapeutic wound healing composition

with the mammalian cells.

120 In a fourth aspect of Embodiment One (I.D), the invention is directed to a method for preventing and reducing injury to mammalian cells, and increasing the resuscitation rate of injured mammalian cells, which comprises the steps of (A) providing a therapeutic wound healing composition which comprises (a) lactate selected from the group consisting of lactic acid, pharmaceutically acceptable salts of lactic acid, and mixtures thereof, (b) an antioxidant, and (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the resuscitation of injured mammalian cells, and (B) contacting the therapeutic wound healing composition with the mammalian cells.

121 In a preferred embodiment, the invention is directed to a method for healing a wound in a mammal which comprises the steps of:

122 (A) providing a therapeutic wound healing composition (I.A) which comprises:

123 (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

124 (b) an antioxidant; and

125 (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the resuscitation of injured mammalian cells; and

126 (B) contacting the therapeutic wound healing composition with the wound.

127 The types of wounds which may be healed using the wound healing compositions of Embodiment One (I.A-D) of the present invention are those which result from an injury which causes epidermal damage such as incisions, wounds in which the skin is broken by a cutting instrument, and lacerations, wounds in which the skin is broken by a dull or blunt instrument. The therapeutic compositions may also be used to treat various dermatological disorders such as hyperkeratosis, photo-aging, burns, donor site wounds from skin transplants, ulcers (cutaneous, decubitus, venous stasis, and diabetic), psoriasis, skin rashes, and sunburn photoreactive processes. The topical therapeutic compositions may also be used orally in the form of a mouth wash or spray to protect and accelerate the healing of injured oral tissue such as mouth sores and burns. The topical therapeutic compositions may further be used in ophthalmological preparations to treat wounds such as those which result from corneal ulcers, radialkeratotomy, corneal transplants, epikeratophakia and other surgically induced wounds in the eye. The topical therapeutic compositions may in addition be used in anorectal creams and suppositories to treat such conditions as pruritus and, proctitis, anal fissures, and hemorrhoids. In a preferred embodiment, the therapeutic compositions are used to treat wounds such as incisions and lacerations.

128 The wound healing compositions of Embodiment One (I.A-D) of the present invention may be utilized in topical products, ingestible products, and tissue culture medium to protect mammalian cells and increase the resuscitation rate of injured mammalian cells. For example, the therapeutic wound healing compositions may be used in topical skin care products to protect and increase the resuscitation rate of skin tissue such as in the treatment of various dermatological disorders such as hyperkeratosis, photo-aging, and sunburn photoreactive processes. Injury to skin can occur for a variety of reasons. Injury often occurs to individuals who wash their hands often, to individuals who are exposed to stressful environmental conditions (overexposure to sun or chemicals), or to the elderly or individuals with an underlining disease. The addition of the wound healing compositions of the present invention to a lotion provides a source of antioxidants to the skin which would protect the skin from the harmful effects of UV light, chemicals, and severe drying. The wound healing compositions can be used for the following indications: a) Moisturizing and protecting; b) Healing dry cracked skin; c) Treating irritated skin such as diaper rash; d) Healing severe dry skin due to other diseases (venous

dermatitis); e) Treating psoriasis and other hyperproliferative diseases; f) Protecting skin from UV light damage (antioxidant skin replacement); g) Treating seborrheic conditions; and h) Treating shaving wounds in an after shave lotion.

129 The topical therapeutic wound healing compositions may also be used orally in the form of a mouth wash or spray to protect and accelerate the healing of injured oral tissue such as mouth sores and bums. The topical therapeutic wound healing compositions may further be used in ophthalmological preparations such as eye care products to neutralize hydrogen peroxide used in the cleaning of contact lenses. The topical therapeutic wound healing compositions may in addition be used in anorectal creams and suppositories to treat such conditions as pruritus and, proctitis, anal fissures, and hemorrhoids. Initially as white blood cells enter a wound site, the cells release oxygen radicals, depleting the andoxidants at the wound site, thus impairing the healing process. Incorporating the wound healing compositions of the present invention into a wound healing formulation would facilitate healing by providing the site with usable antioxidants, and a source of fatty acids needed for membrane repair. The wound healing compositions can be used for the following indications: a) Healing of cuts and scrapes; b) Burns (heals burns with less scaring and scabbing); c) Decubitus ulcers; d) Bed sores, pressure ulcers; e) Fissures, Hemorrhoids; f) Use in combination with immunostimulators (simulated healing in healing deficient people); g) Post surgical wounds; h) Bandages; i) Diabetic ulcers; j) Venous ulceration; and k) Use in combination with wound cleansing agents.

130 The therapeutic wound healing compositions may also be used in ingestible products to protect and increase the resuscitation rate of erosions, stomach ulcers, and hemorrhages in the gastric mucosa. Other ingestible therapeutic products include: stroke medications; autoimmune disease medications; arthritis medications; ulcer medications; cancer medications (cytotoxic agents); heart medication to improve regional ventricular function and restore normal heart rate and pressure functions; lung medication to repair injured tissue; liver medication to suppress lipogenesis of alcoholic origin and prevent hepatic steatosis; kidney medication to suppress urinary calculi (kidney stones); detoxification medication to antagonize heavy metal poisoning, cyanide poisoning, sodium sulfide poisoning, other types of poisoning, and reduce and neutralize the production of oxygen radicals which produces injury to tissue, to protect and further enhance the resuscitation rate of the injured mammalian cells. The therapeutic wound healing compositions may be used in ingestible products to treat inflammatory diseases such as hepatitis, gastritis, colitis, esophagitis, arthritis, and pancreatitis.

131 The therapeutic wound healing compositions of the present invention may also be used in tissue culture media and organ transplant media to prevent and reduce injury to mammalian cells and increase the resuscitation rate of injured mammalian cells. Tissue cultures and transplant organs encounter reactive oxygen species generated in the culture media by the injured cells. Organs particularly susceptible to oxidative damage during transport and transplantation due to reperfusion injury following ischemia are comeas, livers, hearts, and kidneys. The therapeutic wound healing compositions may be usefid to abrogate reperfusion injury to such transplant organs.

132 In a specific embodiment, the invention is directed to a method for preserving mammalian cells in a culture medium which comprises the steps of:

133 (A) providing a therapeutic wound healing composition selected from the group of consisting of:

134 (I.A)

135 (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

136 (b) an antioxidant; and

137 (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids

are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells;

138 (I.B)

139 (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

140 (b) lactate selected from the group consisting of lactic acid, pharmaceutically acceptable salts of lactic acid, and mixtures thereof; and

141 (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells;

142 (I.C)

143 (a) an antioxidant; and

144 (b) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells;

145 (I.D)

146 (a) lactate selected from the group consisting of lactic acid, pharmaceutically acceptable salts of lactic acid, and mixtures thereof;

147 (b) an antioxidant; and

148 (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells; and

149 (b) an antioxidant; and

150 (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the resuscitation of injured mammalian cells;

151 (B) providing mammalian cells in a culture medium; and

152 (C) contacling the therapeutic wound healing composition from step (A) with the mammalian cells in the culture medium from step (B).

153 D. Formulations Of The Therapeutic Wound Healing Compositions Of Embodiment One (I.A-D)

154 Once prepared, the inventive therapeutic wound healing compositions of Embodiment One (I.A-D) may be stored for future use or may be formulated in effective amounts with pharmaceutically acceptable carriers to prepare a wide variety of pharmaceutical compositions. Examples of pharmaceutically acceptable carriers are pharmaceutical appliances, topical vehicles (non-oral and oral), and ingestible vehicles.

155 Examples of pharmaceutical appliances are sutures, staples, gauze, bandages, burn dressings, artificial skins, liposome or micell formulations, microcapsules, aqueous vehicles for soaking gauze dressings, and the like, and mixtures thereof. Non-oral topical compositions employ non-oral topical vehicles, such as creams, gels formulations, foams, ointments and sprays, salves, and films, which are intended to be applied to the skin or body cavity and are not intended to be taken by mouth. Oral topical compositions employ oral vehicles, such as mouthwashes, rinses, oral sprays, suspensions, and dental gels, which are intended to be taken by mouth but are not intended to be ingested. Ingestible compositions employ ingestible or partly ingestible vehicles such as confectionery bulking agents which include hard and soft

confectionery such as lozenges, tablets, toffees, nougats, suspensions, chewy candies, and chewing gums.

156 In one form of the invention, the therapeutic wound healing composition is incorporated into a pharmaceutical appliance which may be in the form of sutures, staples, gauze, bandages, burn dressings, artificial skins, liposome or micell formulations, microcapsules, aqueous vehicles for soaking gauze dressings, and the like, and mixtures thereof. A variety of traditional ingredients may optionally be included in the pharmaceutical composition in effective amounts such as buffers, preservatives, tonicity adjusting agents, antioxidants, polymers for adjusting viscosity or for use as extenders, and excipients, and the like. Specific illustrative examples of such traditional ingredients include acetate and borate buffers; thimerosal, sorbic acid, methyl and propyl paraben and chlorobutanol preservatives; sodium chloride and sugars to adjust the tonicity; and excipients such as marmitol, lactose and sucrose. Other conventional pharmaceutical additives known to those having ordinary skill in the pharmaceutical arts may also be used in the pharmaceutical composition.

157 In accordance with this invention, therapeutically effective amounts of the therapeutic wound healing compositions of the present invention may be employed in the pharmaceutical appliance. These amounts are readily determined by those skilled in the an without the need for undue experimentation. The exact amount of the therapeutic wound healing composition employed is subject to such factors as the type and concentration of the therapeutic wound healing composition and the type of pharmaceutical appliance employed. Thus, the amount of therapeutic wound healing composition may be varied in order to obtain the result desired in the final product and such variations are within the capabilities of those skilled in the an without the need for undue experimentation. In a preferred embodiment, the pharmaceutical composition will comprise the therapeutic wound healing composition in an amount from about 0.1% to about 5%, by weight of the pharmaceutical composition. In a more preferred embodiment, the pharmaceutical composition will comprise the therapeutic wound healing composition in an amount from about 0.1% to about 3%, by weight of the pharmaceutical composition. In a most preferred embodiment, the pharmaceutical composition will comprise the therapeutic wound healing composition in an amount from about 0.1% to about 1%, by weight of the pharmaceutical composition.

158 The present invention extends to methods for making the pharmaceutical compositions. In general, a pharmaceutical composition is made by contacting a therapeutically effective amount of a therapeutic wound healing composition with a pharmaceutical appliance and the other ingredients of the final desired pharmaceutical composition. The therapeutic wound healing composition may be in a solvent and may be absorbed onto a pharmaceutical appliance.

159 Other ingredients will usually be incorporated into the composition as dictated by the nature of the desired composition as well known by those having ordinary skill in the art. The ultimate pharmaceutical compositions are readily prepared using methods generally known in the pharmaceutical arts.

160 In another form of the invention, the therapeutic wound healing composition is incorporated into a non-oral topical vehicle which may be in the form of a cream, gel, foam, ointment, spray, and the like. Typical non-toxic non-oral topical vehicles known in the pharmaceutical arts may be used in the present invention. The preferred non-oral topical vehicles are water and pharmaceutically acceptable water-miscible organic solvents such as ethyl alcohol, isopropyl alcohol, propylene glycol, glycerin, and the like, and mixtures of these solvents. Water-alcohol mixtures are particularly preferred and are generally employed in a weight ratio from about 1:1 to about 20:1, preferably from about 3:1 to about 20:1, and most preferably from about 3:1 to about 10:1, respectively.

161 The non-oral topical therapeutic wound healing compositions may also contain conventional additives employed in those products. Conventional additives include humectants, emollients, lubricants, stabilizers, dyes, and perfumes, providing the additives do not interfere with the therapeutic properties of the

therapeutic wound healing composition.

162 Suitable humectants useful in the non-oral topical therapeutic wound healing compositions include glycerin, propylene glycol, polyethylene glycol, sorbitan, fructose, and the like, and mixtures thereof. Humectants, when employed, may be present in amounts from about 10% to about 20%, by weight of the topical therapeutic wound healing composition.

163 The coloring agents (colors, colorants) useful in the non-oral topical therapeutic wound healing composition are used in amounts effective to produce the desired color. These coloring agents include pigments which may be incorporated in amounts up to about 6% by weight of the non-oral topical therapeutic wound healing composition. A preferred pigment, titanium dioxide, may be incorporated in amounts up to about 2%, and preferably less than about 1%, by weight of the non-oral topical therapeutic wound healing composition. The coloring agents may also include natural food colors and dyes suitable for food, drug and cosmetic applications. These coloring agents are known as F.D.& C. dyes and lakes. The materials acceptable for the foregoing uses are preferably water-soluble. Illustrative nonlimiting examples include the indigoid dye known as F.D.& C. Blue No.2, which is the disodium salt of 5,5-indigotindisulfonic acid. Similarly, the dye known as F.D.& C. Green No.1 comprises a triphenylmethane dye and is the monosodium salt of 4-[4-(N-ethyl-p-sulfoniumbenzylamino) diphenylmethylene]-[1-(N-ethyl-N-p-sulfoniumbenzyl)-delta-2,5-cyclohexadie neimine]. A full recitation of all F.D.& C. coloring agents and their corresponding chemical structures may be found in the Kirk-Othmer Encyclopedia of Chemical Technology, 3rd Edition, in volume 5 at pages 857-884, which text is incorporated herein by reference.

164 In accordance with this invention, therapeutically effective amounts of the therapeutic wound healing compositions of the present invention may be admixed with a non-oral topical vehicle to form a topical therapeutic wound healing composition. These amounts are readily determined by those skilled in the art without the need for undue experimentation. In a preferred embodiment, the non-oral topical therapeutic wound healing compositions will comprise the therapeutic wound healing composition in an amount from about 0.1% to about 10% and a non-oral topical vehicle in a quantity sufficient to bring the total amount of composition to 100%, by weight of the non-oral topical therapeutic wound healing composition. In a more preferred embodiment, the non-oral topical therapeutic wound healing compositions will comprise the therapeutic wound healing composition in an amount from about 0.1% to about 5%, and in a most preferred embodiment, the non-oral topical therapeutic wound healing compositions will comprise the therapeutic wound healing composition in an amount from about 0.1% to about 2%, and a non-oral topical vehicle in a quantity sufficient to bring the total amount of composition to 100%, by weight of the non-oral topical therapeutic wound healing composition.

165 The present invention extends to methods for preparing the non-oral topical therapeutic wound healing compositions. In such a method, the non-oral topical therapeutic wound healing composition is prepared by admixing a therapeutically effective amount of the therapeutic wound healing composition of the present invention and a non-oral topical vehicle. The final compositions are readily prepared using standard methods and apparatus generally known by those skilled in the pharmaceutical arts. The apparatus useful in accordance with the present invention comprises mixing apparatus well known in the pharmaceutical arts, and therefore the selection of the specific apparatus will be apparent to the artisan.

166 In another form of the invention, the therapeutic wound healing composition is incorporated into an oral topical vehicle which may be in the form of a mouthwash, rinse, oral spray, suspension, dental gel, and the like. Typical non-toxic oral vehicles known in the pharmaceutical arts may be used in the present invention. The preferred oral vehicles are water, ethanol, and water-ethanol mixtures. The waterethanol mixtures are generally employed in a weight ratio from about 1:1 to about 20:1, preferably from about 3:1 to about

20:1, and most preferably from about 3:1 to about 10:1, respectively. The pH value of the oral vehicle is generally from about 4 to about 7, and preferably from about 5 to about 6.5. An oral topical vehicle having a pH value below about 4 is generally irritating to the oral cavity and an oral vehicle having a pH value greater than about 7 generally results in an unpleasant mouth feel.

167 The oral topical therapeutic wound healing compositions may also contain conventional additives normally employed in those products. Conventional additives include a fluorine providing compound, a sweetening agent, a flavoring agent, a coloring agent, a humectant, a buffer, and an emulsifier, providing the additives do not interfere with the therapeutic properties of the therapeutic wound healing composition.

168 The coloring agents and humectants, and the amounts of these additives to be employed, set out above as useful in the non-oral topical therapeutic wound healing composition may be used in the oral topical therapeutic wound healing composition.

169 Fluorine providing compounds may be fully or slightly water soluble and are characterized by their ability to release fluoride ions or fluoride containing ions in water and by their lack of reaction with other components in the composition. Typical fluorine providing compounds are inorganic fluoride salts such as water-soluble alkali metal, alkaline earth metal, and heavy metal salts, for example, sodium fluoride, potassium fluoride, ammonium fluoride, cuprous fluoride, zinc fluoride, stannic fluoride, stannous fluoride, barium fluoride, sodium fluorosilicate, ammonium fluorosilicate, sodium fluorozirconate, sodium monofluorophosphate, aluminum mono- and di-fluorophosphates and fluorinated sodium calcium pyrophosphate. Alkali metal fluorides, tin fluoride and monofluorophosphates, such as sodium and stannous fluoride, sodium monofluorophosphate and mixtures thereof, are preferred.

170 The amount of fluorine providing compound present in the present oral topical therapeutic wound healing composition is dependent upon the type of fluorine providing compound employed, the solubility of the fluorine compound, and the nature of the final oral therapeutic wound healing composition. The amount of fluorine providing compound used must be a nontoxic amount. In general, the fluorine providing compound when used will be present in an amount up to about 1%, preferably from about 0.001% to about 0.1%, and most preferably from about 0.001% to about 0.05%, by weight of the oral topical therapeutic wound healing composition.

171 When sweetening agents (sweeteners) are used, those sweeteners well known in the art, including both natural and artificial sweeteners, may be employed. The sweetening agent used may be selected from a wide range of materials including water-soluble sweetening agents, water-soluble artificial sweetening agents, water-soluble sweetening agents derived from naturally occurring water-soluble sweetening agents, dipeptide based sweetening agents, and protein based sweetening agents, including mixtures thereof. Without being limited to particular sweetening agents, representative categories and examples include:

172 (a) water-soluble sweetening agents such as monosaccharides, disaccharides and polysaccharides such as xylose, ribose, glucose (dextrose), mannose, galactose, fructose (levulose), sucrose (sugar), maltose, invert sugar (a mixture of fruclose and glucose derived from sucrose), partially hydrolyzed starch, corn syrup solids, dihydrochalcones, monellin, steviosides, and glycyrrhizin, and mixtures thereof;

173 (b) water-soluble artificial sweeteners such as soluble saccharin salts, i.e., sodium or calcium saccharin salts, cyclamate salts, the sodium, ammonim or calcium salt of 3,4-dihydro-6-methyl-1,2,3-oxathiazine-4-one-2,2-dioxide, the potassium salt of 3,4-dihydro-6-methyl-1,2,3-oxathiazine-4-one-2,2-dioxide (Acesulfame-K), the free acid form of saccharin, and the like;

174 (c) dipeptide based sweeteners, such as L-aspartic acid derived sweeteners, such as L-aspartyl-L-phenylalanine methyl ester (Aspartame) and materials described

in U.S. Pat. No. 3,492,131,
L-Alpha-aspartyl-N-(2,2,4,4-tetramethyl-3-thietanyl)-D-alanin-amide hydrate (Alitame), methyl esters of L-aspartyl-L-phenylglycerine andL-aspartyl-L-2,5-dihydrophenyl-glycine,
L-aspartyl-2,5-dihydro-L-phenylalanine; L-aspartyl-L-(1-cyclohexen)-alanine, and the like;

175 (d) water-soluble sweeteners derived from naturally occurring water-soluble sweeteners, such as chlorinated derivatives of ordinary sugar (sucrose), e.g., chlorodeoxysugar derivatives such as derivatives of chlorodeoxysucrose or chlorodeoxygalactosucrose, known, for example, under the product designation of Sucralose; examples ofchlorodeoxysucrose and chlorodeoxygalacto-sucrose derivatives include but are not limited to: 1-chloro-1'-deoxysucrose;
4-chloro-4-deoxy-Alpha-D-galacto-pyranosyl-Alpha-D-fructofuranoside, or
4-chloro-4-deoxygalactosucrose;
4-chloro-4-deoxy-Alpha-D-galacto-pyranosyl-1-chloro-1-deoxy-.beta.-D-fruto
-furanoside, or 4,1'-dichloro-4,1'-dideoxygalactosucrose;
1',6'-dichloro-1',6'-dideoxysucrose;
4-chloro-4-deoxy-Alpha-D-galacto-pyranosyl-1,6-dichloro-1,6-dideoxy-.beta.
-D-fructo-furanoside, or 4,1',6'-trichloro-4,1',6'-trideoxygalacto-sucrose;
4,6-dichloro-4,6-dideoxy-Alpha-D-galacto-pyranosyl-6-chloro-6-deoxy-.beta.
-D-fructofumnoside, or 4,6,6'-trichloro-4,6,6'-trideoxygalactosucrose;
6,1',6'-trichloro-6,1',6'-trideoxysucrose;
4,6-dichloro-4,6-dideoxy-Alpha-D-galacto-pyranosyl-1,6-dichloro-1,6-di-deo
xy-.beta.-D-fructofuranoside, or
4,6,1',6'-tetrachloro-4,6,1',6'-tetradeoxygalacto-sucrose; and
4,6,1',6'-tetrachloro-4,6,1',6'-tetradeoxy-sucrose; and

176 (e) protein based sweeteners such as thaumaoccous danielli (Thaumatin I and 1I).

177 In general, an effective amount of sweetening agent is utilized to provide the level of sweetness desired in the particular oral topical therapeutic wound healing composition, and this amount will vary with the sweetener selected and the final oral therapeutic product desired. The amount of sweetener normally present is in the range from about 0.0025% to about 90%, by weight of the oral topical therapeutic wound healing composition, depending upon the sweetener used. The exact range of amounts for each type of sweetener is well known in the art and is not the subject of the present invention.

178 The flavoring agents (flavors, flavorants) which may be used include those flavors known to the skilled artisan, such as natural and artificial flavors. Suitable flavoring agents include mints, such as peppermint, citrus flavors such as orange and lemon, artificial vanilla, cinnamon, various fruit flavors, both individual and mixed, and the like.

179 The amount of flavoring agent employed in the oral topical therapeutic wound healing composition is normally a matter of preference subject to such factors as the type of final oral therapeutic wound healing composition, the individual flavor employed, and the strength of flavor desired. Thus, the amount of flavoring may be varied in order to obtain the result desired in the final product and such variations are within the capabilities of those skilled in the art without the need for undue experimentation. The flavoring agents, when used, are generally utilized in amounts that may, for example, range in amounts from about 0.05% to about 6%, by weight of the oral topical therapeutic wound healing composition.

180 Suitable buffer solutions useful in the non-oral topical therapeutic wound healing compositions include citric acid-sodium citrate solution, phosphoric acid-sodium phosphate solution, and acetic acid-sodium acetate solution in amounts up to about 1%, and preferably from about 0.05% to about 0.5% by weight of the oral topical therapeutic wound healing composition.

181 In accordance with this invention, therapeutically effective amounts of the therapeutic wound healing compositions of the present invention may be admixed with an oral topical vehicle to form a topical therapeutic wound healing

composition. These amounts are readily determined by those skilled in the art without the need for undue experimentation. In a preferred embodiment, the oral topical therapeutic wound healing compositions will comprise the therapeutic wound healing composition in an amount from about 0.1% to about 10% and a oral topical vehicle in a quantity sufficient to bring the total amount of composition to 100%, by weight of the oral topical therapeutic wound healing composition. In a more preferred embodiment, the oral topical therapeutic wound healing compositions will comprise the therapeutic wound healing composition in an amount from about 0.1% to about 5%, and in a most preferred embodiment, the oral topical therapeutic wound healing compositions will comprise the therapeutic wound healing composition in an amount from about 0.1% to about 2%, and a oral topical vehicle in a quantity sufficient to bring the total amount of composition to 100%, by weight of the oral topical therapeutic wound healing composition.

182 The present invention extends to methods for preparing the oral topical therapeutic wound healing compositions. In such a method, the oral topical therapeutic wound healing composition is prepared by admixing a therapeutically effective amount of the therapeutic wound healing composition of the present invention and an oral topical vehicle. The final compositions are readily prepared using standard methods and apparatus generally known by those sldlled in the pharmaceutical arts. The apparatus useful in accordance with the present invention comprises mixing apparatus well known in the pharmaceutical arts, and therefore the selection of the specific apparatus will be apparent to the artisan.

183 In a preferred embodiment, an oral topical therapeutic wound healing composition is made by first dissolving coloring agents, sweetening agents, and similar additives in water. The therapeutic wound healing composition is then admixed with the aqueous solution. Then sufficient water or ethanol, or mixtures of water and ethanol, are added to the solution with mixing until the final solution volume is reached. In a more preferred embodiment, the therapeutic wound healing composition is added to the solution as the final ingredient. The final oral topical therapeutic wound healing compositions are readily prepared using methods generally known in the pharmaceutical arts.

184 The oral therapeutic wound healing composition may also be in the form of dental gel. As used herein, the term "gel" means a solid or semisolid colloid which contains considerable quantities of water. The colloid particles in a gel are linked together in a coherent meshwork which immobflizes the water contained inside the meshwork.

185 The dental gel compositions of the present invention may contain the conventional additives set out above for oral topical therapeutic wound healing compositions such as mouthwashes, rinses, oral sprays, and suspensions and, in addition, may contain additional additives such as a polishing agent, a desensitizing agent, and the like, providing the additional additives do not interfere with the therapeutic properties of the therapeutic wound healing composition.

186 In a dental gel composition, the oral vehicle generally comprises water, typically in an amount from about 10% to about 90%, by weight of the dental gel composition. Polyethylene glycol, propylene glycol, glycerin, and mixtures thereof may also be present in the vehicle as humectants or binders in amounts from about 18% to about 30%, by weight of the dental gel composition. Particularly preferred oral vehicles comprise mixtures of water with polyethylene glycol or water with glycerin and polypropylene glycol.

187 The dental gels of the present invention include a gelling agent (thickening agent) such as a natural or synthetic gum or gelatin. Gelling agents such as hydroxyethyl cellulose, methyl cellulose, glycerin, carboxypolymethylene, and gelatin and the like, and mixtures thereof may be used. The preferred gelling agent is hydroxyethyl cellulose. Gelling agents may be used in amounts from about 0.5% to about 5%, and preferably from about 0.5% to about 2%, by weight of the dental gel composition.

188 The dental gel compositions of the present invention may also include a polishing agent. In clear gels, a polishing agent of colloidal silica and/or alkali metal aluminosilicate complexes is preferred since these materials have refractive indices close to the refractive indices of the gelling systems commonly used in dental gels. In non-clear gels, a polishing agent of calcium carbonate or calcium dihydrate may be used. These polishing agents may be used in amounts up to about 75%, and preferably in amounts up to about 50%, by weight of the dental gel composition.

189 The dental gel may also contain a desensitizing agent such as a combination of citric acid and sodium citrate. Citric acid may be used in an amount from about 0.1% to about 3%, and preferably from about 0.2% to about 1%, by weight, and sodium citrate may be used in an amount from about 0.3% to about 9%, and preferably from about 0.6% to about 3%, by weight of the dental gel composition.

190 In accordance with this invention, therapeutically effective amounts of the therapeutic wound healing compositions of the present invention may be admixed into the dental gel compositions. These amounts are readily determined by those skilled in the art without the need for undue experimentation. In a preferred embodiment, the dental gel compositions will comprise the therapeutic wound healing composition in an amount from about 0.1% to about 10% and an oral topical vehicle in a quantity sufficient to bring the total amount of composition to 100%, by weight of the dental gel composition. In a more preferred embodiment, the dental gel compositions will comprise the therapeutic wound healing composition in an amount from about 0.1% to about 5%, and in a most preferred embodiment, the dental gel compositions will comprise the therapeutic wound healing composition in an amount from about 0.1% to about 2%, and an oral topical vehicle in a quantity sufficient to bring the total amount of composition to 100%, by weight of the dental gel composition.

191 The present invention extends to methods for preparing the therapeutic dental gel compositions. In such a method, the dental gel composition is prepared by admixing a therapeutically effective amount of the therapeutic wound healing composition of the present invention and an oral topical vehicle. The final compositions are readily prepared using methods generally known by those skilled in the dental and pharmaceutical arts. The apparatus useful in accordance with the present invention comprises mixing apparatus well known in the pharmaceutical arts, and therefore the selection of the specific apparatus will be apparent to the artisan.

192 In a preferred embodiment, a therapeutic dental gel composition is made by first dispersing a gelling agent in a humectant or water, or a mixture of both, then admixing to the dispersion an aqueous solution of the water-soluble additives such as the fluorine providing compound, sweeteners and the like, then adding the polishing agent, and lastly admixing the flavoring agent and the therapeutic wound healing composition. The final gel mixture is then tubed or otherwise packaged. The liquids and solids in a gel product are proportioned to form a creamy or gelled mass which is extrudable from a pressurized container or from a collapsible tube. The final therapeutic wound healing compositions are readily prepared using methods generally known in the pharmaceutical arts.

193 In yet another form of the invention, the therapeutic wound healing composition is incorporated into an ingestible vehicle. The ingestible vehicle may be a confectionery bulking agent in the form of lozenges, tablets, toffees, nougats, suspensions, chewy candies, chewing gums, and the like. The pharmaceutically acceptable carriers may be prepared from a wide range of materials including, but not limited to, diluents, binders and adhesives, lubricants, disintegrants, coloring agents, bulking agents, flavoring agents, sweetening agents and miscellaneous materials such as buffers and adsorbents that may be needed in order to prepare a particular therapeutic confection.

194 The preparation of confectionery formulations is historically well known and has changed little through the years. Confectionery items have been classified as either "hard" confectionery or "soft" confectionery. The therapeutic wound

healing compositions of the present invention can be incorporated into confectionery compositions by admixing the inventive composition into conventional hard and soft confections.

195 As used herein, the term confectionery material means a product containing a bulking agent selected from a wide variety of materials such as sugar, corn syrup, and in the case of sugarless bulking agents, sugar alcohols such as sorbitol and mannitol and mixtures thereof. Confectionery material may include such exemplary substances as lozenges, tablets, toffee, nougat, suspensions, chewy candy, chewing gum and the like. The bulking agent is present in a quantity sufficient to bring the total amount of composition to 100%. In general, the bulking agent will be present in amounts up to about 99.98%, preferably in amounts up to about 99.9%, and more preferably in amounts up to about 99%, by weight of the ingestible therapeutic wound healing composition.

196 Lozenges are flavored medicated dosage forms intended to be sucked and held in the mouth. Lozenges may be in the form of various shapes such as flat, circular, octagonal and biconvex forms. The lozenge bases are generally in two forms: hard boiled candy lozenges and compressed tablet lozenges.

197 Hard boiled candy lozenges may be processed and formulated by conventional means. In general, a hard boiled candy lozenge has a base composed of a mixture of sugar and other carbohydrate bulking agents kept in an amorphous or glassy condition. This amorphous or glassy form is considered a solid syrup of sugars generally having from about 0.5% to about 1.5% moisture. Such materials normally contain up to about 92% corn syrup, up to about 55% sugar and from about 0.1% to about 5% water, by weight of the final composition. The syrup component is generally prepared from corn syrups high in fmctose, but may include other materials. Further ingredients such as flavoring agents, sweetening agents, acidulants, coloring agents and the like may also be added.

198 Boiled candy lozenges may also be prepared from non-fermentable sugars such as sorbitol, marmitol, and hydrogenated corn syrup. Typical hydrogenated corn syrups are Lycasin, a commercially available product manufactured by Roquette Corporation, and Hystar, a commercially available product manufactured by Lonza, Inc. The candy lozenges may contain up to about 95% sorbitol, a mixture of sorbitol and mannitol in a ratio from about 9.5:0.5 up to about 7.5:2.5, and hydrogenated corn syrup up to about 55%, by weight of the solid syrup component.

199 Boiled candy lozenges may be routinely prepared by conventional methods such as those involving fire cookers, vacuum cookers, and scraped-surface cookers also referred to as high speed atmospheric cookers.

200 Fire cookers involve the traditional method of making a boiled candy lozenge base. In this method, the desired quantity of carbohydrate big agent is dissolved in water by heating the agent in a kettle until the bulking agent dissolves. Additional bulking agent may then be added and cooking continued until a final temperature of 145.degree. C. to 156.degree. C. is achieved. The batch is then cooled and worked as a plastic-like mass to incorporate additives such as flavors, colorants and the like.

201 A high-speed atmospheric cooker uses a heat-exchanger surface which involves spreading a film of candy on a heat exchange surface, the candy is heated to 165.degree. C. to 170.degree. C. in a few minutes. The candy is then rapidly cooled to 100.degree. C. to 120.degree. C. and worked as a plastic-like mass enabling incorporation of the additives, such as flavors, colorants and the like.

202 In vacuum cookers, the carbohydrate bulking agent is boiled to 125.degree. C. to 132.degree. C., vacuum is applied and additional water is boiled off without extra heating. When cooking is complete, the mass is a semi-solid and has a plastic-like consistency. At this point, flavors, colorants, and other additives are admixed in the mass by routine mechanical mixing operations.

203 The optimum mixing required to uniformly mix the flavoring agents, coloring

agents and other additives during conventional manufacturing of boiled candy lozenges is determined by the time needed to obtain a uniform distribution of the materials. Normally, mixing times of from 4 to 10 minutes have been found to be acceptable.

204 Once the boiled candy lozenge has been properly tempered, it may be cut into workable portions or formed into desired shapes. A variety of forming techniques may be utilized depending upon the shape and size of the final product desired. A general discussion of the composition and preparation of hard confections may be found in H. A. Lieberman, Pharmaceutical Dosage Forms: Tablets, Volume I (1980), Marcel Dekker, Inc., New York, N.Y. at pages 339 to 469, which disclosure is incorporated herein by reference.

205 The apparatus useful in accordance with the present invention comprises cooking and mixing apparatus well known in the confectionery manufacturing arts, and therefore the selection of the specific apparatus will be apparent to the artisan.

206 In contrast, compressed tablet confections contain particulate materials and are formed into structures under pressure. These confections generally contain sugars in amounts up to about 95%, by weight of the composition, and typical tablet excipients such as binders and lubricants as well as flavoring agents, coloring agents and the like.

207 In addition to hard confectionery materials, the lozenges of the present invention may be made of soft confectionery materials such as those contained in nougat. The preparation of soft confections, such as nougat, involves conventional methods, such as the combination of two primary components, namely (1) a high boiling syrup such as a corn syrup, hydrogenated starch hydrolysate or the like, and (2) a relatively light textured frappe, generally prepared from egg albumin, gelatin, vegetable proteins, such as soy derived compounds, sugarless milk derived compounds such as milk proteins, and mixtures thereof. The frappe is generally relatively light, and may, for example, range in density from about 0.5 to about 0.7 grams/cc.

208 The high boiling syrup, or "bob syrup" of the soft confectionery is relatively viscous and has a higher density than the frappe component, and frequently contains a substantial amount of carbohydrate bulking agent such as a hydrogenated starch hydrolysate. Conventionally, the final nougat composition is prepared by the addition of the "bob syrup" to the frappe under agitation, to form the basic nougat mixture. Further ingredients such as flavoring agents, additional carbohydrate bulking agent, coloring agents, preservatives, medicaments, mixtures thereof and the like may be added thereafter also under agitation. A general discussion of the composition and preparation of nougat confections may be found in B. W. Minifie, Chocolate, Cocoa and Confectionery: Science and Technology, 2nd edition, AVI Publishing Co., Inc., Westport, Conn. (1980), at pages 424-425, which disclosure is incorporated herein by reference.

209 The procedure for preparing the soft confectionery involves known procedures. In general, the frappe component is prepared first and thereafter the syrup component is slowly added under agitation at a temperature of at least about 65.degree. C., and preferably at least about 100.degree. C. The mixture of components is continued to be mixed to form a uniform mixture, after which the mixture is cooled to a temperature below 80.degree. C., at which point, the flavoring agent may be added. The mixture is further mixed for an additional period until it is ready to be removed and formed into suitable confectionery shapes.

210 The ingestible therapeutic wound healing compositions may also be in the form of a pharmaceutical suspension. Pharmaceutical suspensions of this invention may be prepared by conventional methods long established in the art of pharmaceutical compounding. Suspensions may contain adjunct materials employed in formulating the suspensions of the art. The suspensions of the present invention can comprise:

211 (a) preservatives such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), benzoic acid, ascorbic acid, methyl paraben, propyl paraben, tocopherols, and the like, and mixtures thereof. Preservatives are generally present in amounts up to about 1%, and preferably from about 0.05% to about 0.5%, by weight of the suspension;

212 (b) buffers such as citric acid-sodium citrate, phosphoric acid-sodium phosphate, and acetic acid-sodium acetate in amounts up to about 1%, and preferably from about 0.05% to about 0.5%, by weight of the suspension;

213 (c) suspending agents or thickeners such as cellulosics like methylcellulose, carrageenans like alginic acid and its derivatives, xanthan gums, gelatin, acacias, and microcrystalline cellulose in amounts up to about 20%, and preferably from about 1% to about 15%, by weight of the suspension;

214 (d) antifoaming agents such as dimethyl polysiloxane in amounts up to about 0.2%, and preferably from about 0.01% to about 0.1%, by weight of the suspension;

215 (e) sweetening agents such as those sweeteners well known in the art, including both natural and artificial sweeteners. Sweetening agents such as monosaccharides, disaccharides and polysaccharides such as xylose, ribose, glucose (dextrose), mannose, galactose, fructose (levulose), sucrose (sugar), maltose, invert sugar (a mixture of fructose and glucose derived from sucrose), partially hydrolyzed starch, corn syrup solids, dihydrochalcones, monellin, steviosides, glycyrrhizin, and sugar alcohols such as sorbitol, mannitol, maltitol, hydrogenated starch hydrolysates and mixtures thereof may be utilized in amounts up to about 60%, and preferably from about 20% to about 50%, by weight of the suspension. Water-soluble artificial sweeteners such as soluble saccharin salts, i.e., sodium or calcium saccharin salts, cyclamate salts, the sodium, ammonium or calcium salt of 3,4-dihydro-6-methyl-1,2,3-oxathiazine-4-one-2,2-dioxide, the potassium salt of 3,4-dihydro-6-methyl-1,2,3-oxathiazine-4-one-2,2-dioxide (Acesulfame-K), the free acid form of saccharin, and the like may be utilized in amounts from about 0.001% to about 5%, by weight of the suspension;

216 (f) flavoring agents such as those flavors well known to the skilled artisan, such as natural and artificial flavors and mints, such as peppermint, menthol, citrus flavors such as orange and lemon, artificial vanilla, cinnamon, various fruit flavors, both individual and mixed and the like may be utilized in amounts from about 0.5% to about 5%, by weight of the suspension;

217 (g) coloring agents such as pigments which may be incorporated in amounts up to about 6%, by weight of the suspension. A preferred pigment, titanium dioxide, may be incorporated in amounts up to about 2%, and preferably less than about 1%, by weight of the suspension. The coloring agents may also include natural food colors and dyes suitable for food, drag and cosmetic applications. These colorants are known as F.D.& C. dyes and lakes. The materials acceptable for the foregoing uses are preferably water-soluble. Such dyes are generally present in amounts up to about 0.25%, and preferably from about 0.05% to about 0.2%, by weight of the suspension;

218 (h) decolorizing agents such as sodium metabisulfite, ascorbic acid and the like may be incorporated into the suspension to prevent color changes due to aging. In general, decolorizing agents may be used in amounts up to about 0.25%, and preferably from about 0.05% to about 0.2%, by weight of the suspension; and

219 (i) solubilizers such as alcohol, propylene glycol, polyethylene glycol, and the like may be used to solubilize the flavoring agents. In general, solubilizing agents may be used in amounts up to about 10%, and preferably from about 2% to about 5%, by weight of the suspension.

220 The pharmaceutical suspensions of the present invention may be prepared as follows:

221 (A) admix the thickener with water heated from about 40.degree. C. to about 95.degree. C., preferably from about 40.degree. C. to about 70.degree. C., to form a dispersion if the thickener is not water soluble or a solution if the thickener is water soluble;

222 (B) admix the sweetening agent with water to form a solution;

223 (C) admix the therapeutic wound healing composition with the thickener-water admixture to form a uniform thickener-therapeutic wound healing composition;

224 (D) combine the sweetener solution with the thickener-therapeutic wound healing composition and mix until uniform; and

225 (E) admix the optional adjunct materials such as coloring agents, flavoring agents, decolorants, solubilizers, antifoaming agents, buffers and additional water with the mixture of step (D) to form the suspension.

226 The ingestible therapeutic wound healing compositions of this invention may also be in chewable form. To achieve acceptable stability and quality as well as good taste and mouth feel in a chewable formulation several considerations are important. These considerations include the amount of active substance per tablet, the flavoring agent employed, the degree of compressibility of the tablet and the organoleptie properties of the composition.

227 Chewable therapeutic candy is prepared by procedures similar to those used to make soft confectionery. In a typical procedure, a boiled sugar-corn syrup blend is formed to which is added a frappe mixture. The boiled sugar-corn syrup blend may be prepared from sugar and corn syrup blended in parts by weight ratio of about 90:10 to about 10:90. The sugar-corn syrup blend is heated to temperatures above about 120.degree. C. to remove water and to form a molten mass. The frappe is generally prepared from gelatin, egg albumin, milk proteins such as casein, and vegetable proteins such as soy protein, and the like, which is added to a gelatin solution and rapidly mixed at ambient temperature to form an aerated sponge like mass. The frappe is then added to the molten candy mass and mixed until homogeneous at temperatures between about 65.degree. C. and about 120.degree. C.

228 The ingestible therapeutic wound healing composition of the instant invention can then be added to the homogeneous mixture as the temperature is lowered to about 65.degree. C.-95.degree. C. whereupon additional ingredients can then be added such as flavoring agents and coloring agents. The formulation is further cooled and formed into pieces of desired dimensions.

229 A general discussion of the lozenge and chewable tablet forms of confectionery may be found in H. A. Lieberman and L. Lathman, Pharmaceutical Dosage Forms: Tablets Volume 1, Marcel Dekker, Inc., New York, N.Y. at pages 289 to 466, which disclosure is incorporated herein by reference.

230 In accordance with this invention, therapeutically effective amounts of the therapeutic wound healing compositions of the present invention may be admixed into the hard and soft confectionery products. These amounts are readily determined by those skilled in the art without the need for undue experimentation. In a preferred embodiment, the ingestible therapeutic wound healing composition will comprise the therapeutic wound healing composition in an amount from about 0.1% to about 10% and an ingestible vehicle, that is a pharmaceutically acceptable carrier, in a quantity sufficient to bring the total amount of composition to 100%, by weight the ingestible therapeutic wound healing composition. In a more preferred embodiment, the ingestible composition will comprise the therapeutic wound healing composition in an amount from about 0.1% to about 5%, and in a most preferred embodiment, the ingestible composition will comprise the therapeutic wound healing composition in an amount from about 0.1% to about 2%, and an ingestible vehicle in a quantity sufficient to bring the total amount of composition to 100%, by weight the ingestible therapeutic wound healing composition.

231 The present invention extends to methods of making the ingestible therapeutic wound healing compositions. In such methods, an ingestible therapeutic wound healing composition is prepared by adrnixing a therapeutically effective amount of the therapeutic wound healing composition with a pharmaceutically-acceptable carrier. The apparatus useful in accordance with the present invention comprises mixing and heating apparatus well known in the confectionery arts, and therefore the selection of the specific apparatus will be apparent to the artisan. The final ingestible therapeutic wound healing compositions are readily prepared using methods generally known in the confectionery arts.

232 The therapeutic wound healing compositions may also be incorporated into chewing gums. In this form of the invention, the chewing gum composition contains a gum base, a bulking agent, the inventive therapeutic wound healing composition, and various additives.

233 The gum base employed will vary greatly depending upon various factors such as the type of base desired, the consistency of gum desired and the other components used in the composition to make the final chewing gum product. The gum base may be any water-insoluble gum base known in the art, and includes those gum bases utilized for chewing gums and bubble gums. Illustrative examples of suitable polymers in gum bases include both natural and synthetic elastomers and rubbers. For example, those polymers which are suitable as gum bases include, without limitation, substances of vegetable origin such as chicle, crown gum, nispero, rosadinha, jelutong, perillo, niger gutta, tunu, balata, gutta-percha, lechi-capsi, sorva, gutta kay, mixtures thereof and the like. Synthetic elastomers such as butadiene-styrene copolymers, polyisobutylene, isobutylene-isoprene copolymers, polyethylene, mixtures thereof and the like are particularly useful.

234 The gum base may include a non-toxic vinyl polymer, such as polyvinyl acetate and its partial hydrolysate, polyvinyl alcohol, and mixtures thereof. When utilized, the molecular weight of the vinyl polymer may range from about 2,000 up to and including about 94,000.

235 The amount of gum base employed will vary greatly depending upon various factors such as the type of base used, the consistency of the gum desired and the other components used in the composition to make the final chewing gum product. In general, the gum base will be present in amounts from about 5% to about 94%, by weight of the final chewing gum composition, and preferably in amounts from about 15% to about 45%, and more preferably in amounts from about 15% to about 35%, and most preferably in amounts from about 20% to about 30%, by weight of the final chewing gum composition.

236 The gum base composition may contain conventional elastomer solvents to aid in softening the elastomer base component. Such elastomer solvents may comprise terpinene resins such as polymers of Alpha-pinene or .beta.-pinene, methyl, glycerol or pentaerythritol esters of rosins or modified rosins and gums, such as hydrogenated, dimerized or polymerized rosins or mixtures thereof. Examples of elastomer solvents suitable for use herein include the pentaerythritol ester of partially hydrogenated wood or gum rosin, the pentaerythritol ester of wood or gum rosin, the glycerol ester of wood rosin, the glycerol ester of partially dimerized wood or gum rosin, the glycerol ester of polymerized wood or gum rosin, the glycerol ester of tail oil rosin, the glycerol ester of wood or gum rosin and the partially hydrogenated wood or gum rosin and the partially hydrogenated methyl ester of wood or rosin, mixtures thereof, and the like. The elastomer solvent may be employed in amounts from about 5% to about 75%, by weight of the gum base, and preferably from about 45% to about 70%, by weight of the gum base.

237 A variety of traditional ingredients may be included in the gum base in effective amounts such as plasticizers or softeners such as lanolin, palmitic acid, oleic acid, stearic acid, sodium stearate, potassium stearate, glyceryl triacetate, glyceryl lecithin, glyceryl monostearate, propylene glycol monostearate, acetylated monoglyceride, glycerine, mixtures thereof, and the like may also be incorporated into the gum base to obtain a variety of desirable

textures and consistency properties. Waxes, for example, natural and synthetic waxes, hydrogenated vegetable oils, petroleum waxes such as polyurethane waxes, polyethylene waxes, paraffin waxes, microcrystalline waxes, fatty waxes, sorbitan monostearate, tallow, propylene glycol, mixtures thereof, and the like may also be incorporated into the gum base to obtain a variety of deskable textures and consistency properties. These traditional additional materials are generally employed in amounts up to about 30%, by weight of the gum base, and preferably in amounts from about 3% to about 20%, by weight of the gum base.

238 The gum base may include effective amounts of mineral adjuvants such as calcium carbonate, magnesium carbonate, alumina, aluminum hydroxide, aluminum silicate, talc, tricalcium phosphate, dicalcium phosphate and the like as well as mixtures thereof. These mineral adjuvants may serve as fillers and textural agents. These fillers or adjuvants may be used in the gum base in various amounts. Preferably the amount of filler when used will be present in an amount up to about 60%, by weight of the chewing gum base.

239 The chewing gum base may additionally include the conventional additives of coloring agents, antioxidants, preservatives and the like. For example, titanium dioxide and other dyes suitable for food, drug and cosmetic applications, known as F.D. & C. dyes, may be utilized. An antioxidant such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate, and mixtures thereof, may also be included. Other conventional chewing gum additives known to one having ordinary skill in the chewing gum art may also be used in the chewing gum base.

240 The gum composition may include effective amounts of conventional additives selected from the group consisting of sweetening agents (sweeteners), plasticizers, softeners, emulsifiers, waxes, fillers, bulking agents, mineral adjuvants, flavoring agents (flavors, flavorings), coloring agents (colorants, colorings), antioxidants, acidulants, thickeners, mixtures thereof and the like. Some of these additives may serve more than one purpose. For example, in sugarless gum compositions, the sweetener, e.g., sorbitol or other sugar alcohol or mixtures thereof, may also function as a bulking agent. Similarly, in sugar containing gum compositions, the sugar sweetener can also function as a bulking agent.

241 The plasticizers, softeners, mineral adjuvants, colorants, waxes and antioxidants discussed above as being suitable for use in the gum base may also be used in the gum composition. Examples of other conventional additives which may be used include emulsifiers, such as lecithin and glyceryl monostearate, thickeners, used alone or in combination with other softeners, such as methyl cellulose, alginates, carrageenan, xanthan gum, gelatin, carob, tragacanth, locust bean, and carboxy methyl cellulose, acidulants such as malic acid, adipic acid, citric acid, tamtic acid, fumaric acid, and mixtures thereof, and fillers, such as those discussed above under the category of mineral adjuvants. The fillers when used may be utilized in an amount up to about 60%, by weight of the gum composition.

242 Bulking agents (carriers, extenders) suitable for use in chewing gums include sweetening agents selected from the group consisting of monosaccharides, disaccharides, poly-saccharides, sugar alcohols, and mixtures thereof; polydextrose; maltodextrins; minerals, such as calcium carbonate, talc, titanium dioxide, dicalcium phosphate, and the like. Bulking agents may be used in amounts up to about 90%, by weight of the final gum composition, with amounts from about 40% to about 70%, by weight of the gum composition being preferred, with from about 50% to about 65%, by weight, being more preferred and from about 55% to about 60%, by weight of the chewing gum composition, being most preferred.

243 The sweetening agent used may be selected from a wide range of materials including water-soluble sweeteners, water-soluble artificial sweeteners, water-soluble sweeteners derived from naturally occurring water-soluble sweeteners, dipeptide based sweeteners, and protein based sweeteners, including mixtures thereof. Without being limited to particular sweeteners, representative

categories and examples include:

244 (a) water-soluble sweetening agents such as monosaccharides, disaccharides and polysaccharides such as xylose, ribulose, glucose (dextrose), mannose, galactose, fructose (levulose), sucrose (sugar), maltose, invert sugar (a mixture of fructose and glucose derived from sucrose), partially hydrolyzed starch, corn syrup solids, dihydrochalcones, monellin, steviosides, glycyrrhizin, and sugar alcohols such as sorbitol, mannitol, maltitol, hydrogenareal starch hydrolysates and mixtures thereof;

245 (b) water-soluble artificial sweeteners such as soluble saccharin salts, i.e., sodium or calcium saccharin salts, cyclamate salts, the sodium, ammonim or calcium salt of 3,4-dihydro-6-methyl-1,2,3-oxathiazine-4-one-2,2-dioxide, the potassium salt of 3,4-dihydro-6-methyl-1,2,3-oxathiazine-4-one-2,2-dioxide (Acesulfame-K), the free acid form of saccharin, and the like;

246 (c) dipeptide based sweeteners, such as L-aspartic acid derived sweeteners, such as L-aspartyl-L-phenylalanine methyl ester (Aspartame) and materials described in U.S. Pat. No. 3,492,131, L-Alpha-aspartyl-N-(2,2,4,4-tetramethyl-3-thietanyl)-D-alanin-amide hydrate (Alitame), methyl esters of L-aspartyl- L-phenylglycerine andL-aspartyl-L-2,5-dihydrophenyl-glycine, L-aspartyl-2,5-dihydro-L-phenylalanine; L-aspartyl-L-(1-cyclohexen)-alanine, and the like;

247 (d) water-soluble sweeteners derived from naturally occurring water-soluble sweeteners, such as chlorinated derivatives of ordinary sugar (sucrose), known, for example, under the product designation of Sucralose; and

248 (e) protein based sweeteners such as thaumaoccous danielli (Thaumatin I and II).

249 In general, an effective amount of sweetener is utilized to provide the level of bulk and/or sweetness desired, and this amount will vary with the sweetener selected. This amount of sweetener will normally be present in amounts from about 0.0025% to about 90%, by weight of the gum composition, depending upon the sweetener used. The exact range of amounts for each type of sweetener is well known in the art and is not the subject of the present invention. The amount of sweetener ordinarily necessary to achieve the desired level of sweemess is independent from the flavor level achieved from flavor oils.

250 Preferred sugar base&sweeteners are sugar (sucrose), corn syrup and mixtures thereof. Preferred sugarless sweeteners are the sugar alcohols, artificial sweeteners, dipeptide based sweeteners and mixtures thereof. Preferably, sugar alcohols are used in the sugarless compositions because these sweeteners can be used in amounts which are sufficient to provide bulk as well as the desired level of sweetness. Preferred sugar alcohols are selected from the group consisting of sorbitol, xylitol, maltitol, mannitol, and mixtures thereof. More preferably, sorbitol or a mixture of sorbitol and mannitol is utilized. The gamma form of sorbitol is preferred. An artificial sweetener or dipeptide based sweetener is preferably added to the gum compositions which contain sugar alcohols.

251 The coloring agents useful in the gum compositions are used in amounts effective to produce the desired color. These coloring agents include pigments which may be incorporated in amounts up to about 6% by weight of the gum composition. A preferred pigment, titanium dioxide, may be incorporated in amounts up to about 2%, and preferably less than about 1% by weight of the composition. The colorants may also include natural food colors and dyes suitable for food, drug and cosmetic applications. These colorants are known as F.D.& C. dyes and lakes. The materials acceptable for the foregoing uses are preferably water-soluble. Illustrative nonlimiting examples include the indigoid dye known as F.D.& C. Blue No.2, which is the disodium salt of 5,5-indigotindisulfonic acid. Similarly, the dye known as F.D.& C. Green No. 1 comprises a triphenylmethane dye and is the monosodium salt of 4-[4-(N-ethyl-p-sulfoninmbenzylamino)diphenylmethylene]-[1-(N-ethyl-N-p-su

lfoniumbenzyl)-delta-2,5-cyclohexadieneimine]. A full recitation of all F.D.& C. colorants and their corresponding chemical structures may be found in the Kirk-Othmer Encyclopedia of Chemical Technology, 3rd Edition, in volume 5 at pages 857-884, which text is incorporated herein by reference.

252 Suitable oils and fats usable in gum compositions include partially hydrogenated vegetable or animal fats, such as coconut oil, palm kernel oil, beef tallow, lard, and the like. These ingredients when used are generally present in amounts up to about 7%, by weight, and preferably up to about 3.5%, by weight of the gum composition.

253 In accordance with this invention, therapeutically effective amounts of the therapeutic wound healing compositions of the present invention may be admixed into a chewing gum. These amounts are readily determined by those skilled in the art without the need for undue experimentation. In a preferred embodiment, the final chewing gum composition will comprise the therapeutic wound healing composition in an amount from about 0.1% to about 10% and a chewing gum composition in a quantity sufficient to bring the total amount of composition to 100%, by weight of the chewing gum composition. In a more preferred embodiment, the final chewing gum composition will comprise the therapeutic wound healing composition in an amount from about 0.1% to about 5%, and in a most preferred embodiment, the final chewing gum composition will comprise the therapeutic wound healing composition in an amount from about 0.1% to about 2%, and a chewing gum composition in a quantity sufficient to bring the total amount of composition to 100%, by weight of the chewing gum composition.

254 The present invention extends to methods of making the therapeutic chewing gum compositions. The therapeutic wound healing compositions may be incorporated into an otherwise conventional chewing gum composition using standard techniques and equipment known to those skilled in the art. The apparatus useful in accordance with the present invention comprises mixing and heating apparatus well known in the chewing gum manufacturing arts, and therefore the selection of the specific apparatus will be apparent to the artisan.

255 For example, a gum base is heated to a temperature sufficiently high enough to soften the base without adversely eftcoting the physical and chemical make up of the base. The optimum temperatures utilized may vary depending upon the composition of the gum base used, but such temperatures are readily determined by those skilled in the art without undue experimentation.

256 The gum base is conventionally melted at temperatures that range from about 60.degree. C. to about 120.degree. C. for a period of time sufficient to render the base molten. For example, the gum base may be heated under these conditions for a period of about thirty minutes just prior to being admixed incrementally with the remaining ingredients of the base such as the plasticizer, fillers, the bulking agent and/or sweeteners, the softener and coloring agents to plasticize the blend as well as to modulate the hardness, viscoelasticity and formability of the base. The chewing gum base is then blended with the therapeutic wound healing composition of the present invention which may have been previously blended with other traditional ingredients. Mixing is continued until a uniform mixture of gum composition is obtained. Thereafter the gum composition mixture may be formed into desirable chewing gum shapes.

257 In a specific embodiment, the invention is directed to a therapeutic pharmaceutical composition for preventing and reducing injury to mammalian cells, and increasing the resuscitation rate of injured mammalian cells, which comprises:

258 (A) a therapeutically effective amount of a therapeutic wound healing composition of Embodiment One (I) selected from the group consisting of:

259 (I.A)

260 (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

.

.

261 (b) an antioxidant; and

262 (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells;

263 (I.B)

264 (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

265 (b) lactate selected from the group consisting of lactic acid, pharmaceutically acceptable salts of lactic acid, and mixtures thereof; and

266 (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells;

267 (I.C)

268 (a) an antioxidant; and

269 (b) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells;

270 (I.D)

271 (a) lactate selected from the group consisting of lactic acid, pharmaceutically acceptable salts of lactic acid, and mixtures thereof;

272 (b) an antioxidant; and

273 (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells; and

274 (B) a pharmaceutically acceptable carrier.

275 The pharmaceutically acceptable carrier may be selected from the group consisting of pharmaceutical appliances, topical vehicles, and ingestible vehicle.

276 In another specific embodiment, the invention is directed to a method for preparing a therapeutic pharmaceutical composition for preventing and reducing injury to mammalian cells, and increasing the resuscitation rate of injured mammalian cells, which comprises the steps of:

277 (A) providing a therapeutically effective amount of a therapeutic wound healing composition of Embodiment One (I.A-D) selected from the group consisting of:

278 (I.A)

279 (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

280 (b) an antioxidant; and

281 (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells;

282 (I.B)

283 (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

284 (b) lactate selected from the group consisting of lactic acid, pharmaceutically acceptable salts of lactic acid, and mixtures thereof; and

285 (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells;

286 (I.C)

287 (a) an antioxidant; and

288 (b) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells;

289 (I.D)

290 (a) lactate selected from the group consisting of lactic acid, pharmaceutically acceptable salts of lactic acid, and mixtures thereof;

291 (b) an antioxidant; and

292 (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells; and

293 (B) providing a pharmaceutically acceptable carrier; and

294 (C) admixing the therapeutic wound healing composition from step (A) and the pharmaceutically acceptable carrier from step (B) to form a therapeutic pharmaceutical composition.

295 Throughout this application, various publications have been referenced. The disclosures in these publications are incorporated herein by reference in order to more fully describe the state of the art.

296 The present invention is further illustrated by the following examples which are not intended to limit the effective scope of the claims. All parts and percentages in the examples and throughout the specification and claims are by weight of the final composition unless otherwise specified.

297 E. Examples Of The Therapeutic Wound Healing Compositions Of Embodiment One (I.A-D)

298 Study 1

299 This study demonstrates a comparison of the viability of U937 monocytic cells after exposure of the cells to various antioxidants and combinations of antioxidants. This study also demonstrate a comparison of the levels of hydrogen peroxide produced by U937 monocytic cells and mammalian epidermal keratinocytes after exposure of the cells to various antioxidants and combinations of antioxidants. The results of this study are illustrated in FIGS. 1-4 and examples 1-26 below.

300 Mammalian epidermal keratinocytes and monocytes were employed to examine the ability of various antioxidants to reduce levels of hydrogen peroxide in these cells. Hydrogen peroxide was measured after the cells were exposed to ultraviolet light in the wavelength range from 290 to 320 nm (UV-B) or to the inflammatory compound 12-0-tetradecanoyl-phorbol-13-acetate (TPA). Sodium pyruvate was tested at various concentrations to determine the effect of concentrations of this antioxidant on the hydrogen peroxide production by epidermal cells and monocytes. Magnesium pyruvate, calcium pyruvate, zinc

pyruvate, and combinations of sodium pyruvate with ascorbic acid, lactic acid, and Vitamin E were then tested to determine the effect of these salts and combinations of antioxidants on the hydrogen peroxide production by epidermal cells and monocytes.

301 Mammalian epidermal kemtinocytes were isolated by trypsinization of epithelial sheets and grown in modified basal MCDB 153 medium supplemented with epidermal growth factor, bovine pituitary extract, and hydrocortisone. Cells were maintained in a humidified incubator with 5% carbon dioxide at 37.degree. C. Keratinocytes were seeded in 60 mm culture dishes at a cell density of 3.times.10.sup.5 cells per dish and the cultures were exposed to 1 M.E.D. dose of ultraviolet-B light (100 mJ/cm.sup.2) or treated with 100 ng/ml of TPA.

302 U937 monocytic cells are a cultured cell line grown in RPMI media with 10% fetal calf serum. Cells were maintained in a 60 mm culture dish at 5% carbon dioxide at 37.degree. C. at a seeding density not exceeding 1.times.10.sup.6 cells per dish.

303 Sodium pyruvate, lactic acid, ascorbic acid, and Vitamin E were dissolved in distilled water, with sufficient surfactant. The concentrations of the sodium pyruvate solutions prepared were 1 mM, 10 mM, 50 mM, 100 mM, and 200 mM. The concentrations of the lactic acid solutions prepared were 1.0%, 0.1%, and 0.05%. The concentrations of the ascorbic acid solutions prepared were 1.0%, 0.1%, 0.05%, and 0.025%. The concentrations of the Vitamin E solutions prepared were 1 U, 10 U, 50 U, and 100 U. The test solutions were adjusted to a pH value of 7.4 with 1.0N sodium hydroxide solution and then sterile filtered. The appropriate concentration of test solution or combination of test solutions was added to the cells immediately prior to exposure of the cells to ultraviolet light-B or TPA [100 ng/ml]. Stock solutions were prepared so that the vehicle did not constitute more than 1% of the total volume of the culture media.

304 Intracellular hydrogen peroxide production by mammalian epidermal keratinocytes and U937 monoceytes was measured using dichlorofluorescein diacetate (DCFH-DA, Molecular Probes, Eugene, Oreg.). DCFH-DA is a non-polar nonfluorescent compound that readily diffuses into cells where it is hydrolyzed to the polar non-fluorescent derivative DCFH which then becomes trapped within the cells. In the presence of intracellular hydrogen peroxide, DCFH is oxidized to the highly fluorescent compound DCF. Hence, cellular fluorescence intensity is directly proportional to the level of intracellular hydrogen peroxide produced. Cellular fluorescence intensity can be monitored by fluorimetry and by flow cytometry.

305 Mammalian epidermal keratinocytes and U937 cultured monocytes (1.times.10.sup.6 per dish) were incubated at 37.degree. C. with 5 uM of DCFH-DA. Production of hydrogen peroxide was measured using a Coulter Profile analytical flow cytometer. Linear and log intensity of green fluorescence data was collected. For each analysis, a quantity of 10,000 to 20,000 events was accumulated. Optical alignment for the instrument was performed daily. Coefficients of variation for forward angle light scatter and integrated green fluorescence were generally less than two. Each analysis was repeated three times and the quantitation of fluorescence was expressed in terms of femtomoles (fmol, 10.sup.-15 moles) of DCF oxidized per cell, which is a direct measure of the intracellular hydrogen peroxide produced. Alternatively, in the saturated and unsaturated fatty acid examples in examples 27-52, fluorimetry was used to assess the DCF oxidation per cell.

306 The viability of the U937 monocytic cells after exposure of the cells to various antioxidants for 24 hours was measured. The viability of the cells was determined by exposing the cells to the dye propidium iodide. Permeable cell membranes which absorbed the dye were not considered viable. The viability of the cells was represented as the percentage of cells that excluded propidium iodide. FIG. 1 depicts in bar graph format the viability of U937 monocytic cells after exposure of the cells to no antioxidant (Example 1, control), to sodium pyruvate (Example 2), to ascorbic acid (Example 3), to lactic acid (Example 4), and to Vitamin E (Example 5). FIG. 2 depicts in bar graph format the viability of U937 monocytic cells after exposure of the cells to various combinations of

antioxidants. Specifically, the viability of U937 monocytic cells was measured after exposure to no antioxidant (Example 6, control), to ascorbic acid and lactic acid (Example 7), to ascorbic acid and Vitamin E (Example 8), to sodium pyruvate and ascorbic acid (Example 9), to sodium pyruvate and lactic acid (Example 10), to sodium pyruvate and Vitamin E (Example 11), to lactic acid and Vitamin E (Example 12), and to sodium pyruvate, ascorbic acid, and lactic acid (Example 13).

307 FIG. 1 shows that ascorbic acid is cytotoxic to monocytes at concentrations as low as 0.25%. FIG. 2 shows that the cytotoxicity of ascorbic acid was reversed by the addition of 10 mM of sodium pyruvate. FIGS. 1 and 2 show that the viability rate of 15% to 20% of the cells when treated with ascorbic acid was increased to 95% to 98% upon addition of sodium pyruvate. Lactic acid and Vitamin E did not reverse the cytotoxicity of ascorbic acid.

308 Sodium pyruvate was then tested at various concentrations to determine the effect of concentrations of this antioxidant on the hydrogen peroxide production by epidermal cells and monocytes. Mammalian epidermal keratinocytes and monocytes were exposed to (a) 1 M.E.D. dose of ultraviolet light-B and (b) 100 ng/ml of 12-O-tetradecanoylphorbol-13-acetate (TPA) in the presence of sodium pyruvate at the following concentrations: 200 mM, 100 mM, 50 mM, 10 mM, 1 mM.

309 The optimum concentration of sodium pyruvate to reduce the hydrogen peroxide production by epidermal cells and monocytes was found to be 10 mM. Concentrations of sodium pyruvate of 50 mM and above were cytotoxic to both epidermal keratinocytes and monocytes.

310 Magnesium pyruvate, calcium pyruvate, zinc pyruvate, ascorbic acid, lactic acid, and Vitamin E, and combinations of sodium pyruvate with ascorbic acid, lactic acid, and Vitamin E were then tested to determine the effect of these salts and combinations of antioxidants on the hydrogen peroxide production by epidermal cells and monocytes. The following test solutions were prepared.

311 (a) sodium pyruvate [10 mM];

312 (b) zinc salt [10 mM];

313 (c) magnesium salt [10 mM];

314 (d) calcium salt [10 mM];

315 (e) sodium pyruvate [10 mM] and ascorbic acid [0.025%];

316 (f) sodium pyruvate [10 mM] and lactic acid [0.05%];

317 (g) sodium pyruvate [10 mM], lactic acid, [0.05%], and astorhie acid [0.025%];

318 (h) lactic acid [1.0%, 0.1%, and 0.05%];

319 (i) ascorbic acid [1.0%, 0.1%, 0.05%, and 0.025%];

320 (j) Vitamin E [1 U, 10 U, 50 U, and 100 U]; and

321 (k) vehicle solvent controls.

322 There was no significant difference among the zinc, magnesium, and calcium salts of pyruvic acid on the hydrogen peroxide production by epidermal cells and monocytes. The zinc and calcium salts of pyruvic acid induced differentiation of keratinocytes. For convenience, the sodium salt was used in subsequent tests.

323 The optimum concentration of lactic acid to reduce the hydrogen peroxide production by epidermal cells and monocytes was found to be 0.05%. The optimum concentration of ascorbic acid was found to be 0.025%. The higher concentrations of both of these compounds were found to be cytotoxic to both types of cells. The optimum concentration of Vitamin E was found to be 50 U.

324 FIG. 3 depicts in bar graph format the levels of hydrogen peroxide produced by U937 monocytic cells after exposure of the cells to no antioxidant (Example 14, control), to sodium pyruvate (Example 15), to ascorbic acid (Example 16), to lactic acid (Example 17), and to Vitamin E (Example 18). Sodium pyruvate and Vitamin E significantly reduced the hydrogen peroxide production by monocytes.

325 FIG. 4 depicts in bar graph format the levels of hydrogen peroxide produced by U937 monocytic cells after exposure of the cells to various combinations of antioxidants. Specifically, the levels of hydrogen peroxide produced by U937 monocytic cells were measured after exposure to no antioxidant (Example 19, control), to ascorbic acid and lactic acid (Example 20), to ascorbic acid and Vitamin E (Example 21), to sodium pyruvate and ascorbic acid (Example 22), to sodium pyruvate and lactic acid (Example 23), to sodium pyruvate and Vitamin E (Example 24), to lactic acid and Vitamin E (Example 25), and to sodium pyruvate, ascorbic acid, and lactic acid (Example 26). The combination of lactic acid (0.05%) and Vitamin E (50 U) significantly reduced the hydrogen peroxide production by monocytes.

326 The morphological alterations in epidermal keratinocytes were observed in control cultures and in cultures exposed to ultraviolet-B. Cells in the layer closest to the dermis are basal keratinocytes. These cells proliferate and migrate into the spinous and granular layers of the epidermis where the cells begin to differentiate. The differentiation pattern results in cells enucleating and forming cornified envelopes at the uppermost portion of the epidermis, the statum corneum. The differentiation of keratinocytes is controlled by the levels of calcium, magnesium, and other elements in the medium. Cells in culture systems promoting differentiation appear as an epidermal sheet forming attachments or tight junctions with each other. Keratinocytes that become nonadherent or float in the media were considered responding to a cytotoxic event.

327 The following morphological alterations in the mammalian epidermal keratinocytes were observed for the following control cultures:

328 10 mM Sodium Pyruvate: Tight junctions of cells were formed and the proliferation rate of the cells was higher than the rate of the control cells.

329 0.025% Ascorbic Acid: Cells were floating in a cytotoxic response to ascorbic acid.

330 0.025% Ascorbic acid and 10 mM Sodium Pyruvate: Few tight junctions of cells were observed and cells appeared similar to the cells in the sodium pyruvate culture.

331 0.05% Lactic Acid: Cells appeared dramatically altered as an epidermal sheet and as flat granular cells.

332 0.05% Lactic Acid and 10 mM Sodium Pyruvate: Cells formed an epidermal sheet but appeared smaller than the cell in the lacdc acid culture.

333 50 U Vitamin E: Cells appeared the same as the cells in the control culture.

334 50 U Vitamin E and 10 mM Sodium Pyruvate: Cells increased in number and changed in appearance resembling the cells in the sodium pyruvate culture.

335 The following morphological alterations in the mammalian epidermal keratinocytes were observed for the corresponding cultures exposed to ultraviolet light-B, 100 mJoules, for 24 hours:

336 10 mM Sodium Pyruvate: Cells proliferated more rapidly than the cells in the control culture.

337 0.025% Ascorbic Acid: Cells were nonadherent and floating in a cytotoxic response to ascorbic acid greater than the cytotoxic response of the

corresponding cells without ultraviolet-B light exposure.

338 0.05% Lactic Acid: Cells formed an epidermal sheet and were more granular than cells in the control culture without ultraviolet-B light exposure.

339 50 U Vitamin E: Cell growth was inhibited but cells appeared similar to cells in the control culture without ultraviolet-B light exposure.

340 50 U Vitamin E and 10 mM Sodium Pyruvate: Cells appeared similar to cells in the control culture and proliferated to a greater extent than cells in the control cultures without ultraviolet-B light exposure.

341 Morphological alterations in the U937 monocytic cell line were also observed for control cultures and cultures exposed to ultraviolet light-B, 100 mJoules, for 24 hours. The following compounds and combination of compounds, at the concentrations set out below, significantly inhibited the levels of hydrogen peroxide produced by U937 monocytic cells

Sodium pyruvate at 10 mM and 50 mM;

Vitamin E at 50 U and 100 U; and

Lactic acid at 0.05% and Vitamin E at 50 U.

342 Examples Of The Therapeutic Wound Healing Compositions Of Embodiment One (I.A-D)

343 Study 2

344 This study demonstrates a comparison of the levels of hydrogen peroxide produced by U937 monocytic cells and epidermal keratinocytes after exposure of the cells to various combinations of antioxidants with and without a mixture of saturated and unsaturated fatty acids. The results of this study are illustrated in FIGS. 5-7 and examples 27-52 below.

345 Mammalian epidermal keratinocytes and U937 monocytic cells and the test solutions of sodium pyruvate, lactic acid, ascorbic acid, and Vitamin E were prepared as describe above for Examples 1-26. Intracellular hydrogen peroxide production by the mammalian epidermal keratinocytes and U937 monocytes was also measured as described above.

346 A mixture of fatty acids derived from chicken fat was prepared for addition to the cultured cells by mixing 0.1% of the chicken fat with the culture media. At the temperature of the culture media, 37.degree. C., the chicken fat was miscible. This chicken fat mixture was added to cultures of cells prior to exposure of the cells to ultraviolet-B light or TPA treatment.

347 As set out in examples 1-26, mammalian epidermal keratinocytes and monocytes were exposed to (a) 1 M.E.D. dose of ultraviolet light-B and (b) 100 ng/ml of 12-O-tetradecanoylphorbol-13-acetate in the presence of various antioxidants and combinations of antioxidants with and without a mixture of saturated and unsaturated fatty acids [0.1%, 0.5%, and 1.0% chicken fat].

348 FIG. 5 depicts in bar graph format the levels of hydrogen peroxide produced by U937 monocytic cells after exposure of the cells to various combinations of antioxidants with and without a mixture of saturated and unsaturated fatty acids. Specifically, the levels of hydrogen peroxide produced by U937 monocytic cells were measured after exposure to lactic acid and Vitamin E without fatty acids (Example 27) and with fatty acids (Example 28), to ascorbic acid and lactic acid without fatty acids (Example 29) and with fatty acids (Example 30), and to ascorbic acid and Vitamin E without fatty acids (Example 31) and with fatty acids (Example 32). The ability of the combinations of lactic acid and Vitamin E, ascorbic acid and lactic acid, and ascorbic acid and Vitamin E to reduce the hydrogen peroxide production by monocytes was increased in the

presence of fatty acids. The most effective combination to reduce the hydrogen peroxide production of monocytes was lactic acid (0.05%) and Vitamin E (50 E) in the presence of a mixture of saturated and unsaturated fatty acids (0.5%).

349 FIG. 6 depicts in bar graph format the levels of hydrogen peroxide produced by epidermal keratinocytes after exposure of the cells to various antioxidants with and without a mixture of saturated and unsaturated fatty acids. Specifically, the levels of hydrogen peroxide produced by epidermal keratinocytes were measured after exposure to no antioxidant without fatty acids (Example 33, control) and with fatty acids (Example 34), to sodium pyruvate without fatty acids (Example 35) and with fatty acids (Example 36), to ascorbic acid without fatty acids (Example 37) and with fatty acids (Example 38), to lactic acid without fatty acids (Example 39) and with fatty acids (Example 40), and to Vitamin E without fatty acids (Example 41) and with fatty acids (Example 42). The ability of sodium pyruvate and Vitamin E to reduce the hydrogen peroxide production by epidermal keratinocytes was increased in the presence of fatty acids. The most effective combinations to reduce the hydrogen peroxide production of epidermal keratinocytes were sodium pyruvate in combination with a mixture saturated and unsaturated fatty acids and Vitamin E in combination with a mixture of saturated and unsaturated fatty acids.

350 FIG. 7 depicts in bar graph format the levels of hydrogen peroxide produced by epidermal keratinocytes after exposure of the cells to various combinations of antioxidants with and without a mixture of saturated and unsaturated fatty acids. Specifically, the levels of hydrogen peroxide produced by epidermal keratinocytes were measured after exposure to no antioxidant without fatty acids (Example 43, control) and with fatty acids (Example 44), to sodium pyruvate and ascorbic acid without fatty acids (Example 45) and with fatty acids (Example 46), to sodium pyruvate and lactic acid without fatty acids (Example 47) and with fatty acids (Example 48), to sodium pyruvate and Vitamin E without fatty acids (Example 49) and with fatty acids (Example 50), and to ascorbic acid and Vitamin E without fatty acids (Example 51) and with fatty acids (Example 52). The ability of all combinations of antioxidants to reduce the hydrogen peroxide production by epidermal kemtinocytes was increased in the presence of fatty acids. In order of potency, the most effective combinations to reduce the hydrogen peroxide production of epidermal keratinocytes were sodium pyruvate and Vitamin E, sodium pyruvate and lactic acid, and Vitamin E, each in combination with a mixture of saturated and unsaturated fatty acids (0.5%).

351 Because of the cytotoxicity of cells towards ascorbic acid described above, the ascorbic acid combinations without sodium pyruvate were not considered significantly different from the control test solution.

352 Summary Anaylsis of the Data from Studies 1 and 2

353 Human epidermal keratinocytes were isolated by trypsinization of epithelial sheets and grown in modified base MCDB 153 medium supplemented with epidermal growth factor and bovine pituitary extract. Cells were seeded in culture dishes at a density of 3.times.10.sup.5 /dish. Prior to exposure to UV B light (100 mJ/cm.sup.2) or treatment with 100 ng/ml TPA, the cultures were treated with the appropriate concentration of wound healing components. Intracellular production of hydrogen peroxide was measured using DCFH-DA, a nonpolar compound that readily diffuses into cells, hydrolyzed to a nonpolar derivative. In the presence of intracellular hydrogen peroxide, DCFH is oxidized to a highly fluorescent compound DCF. Thus, cellular fluorescence intensity is directly proportional to levels of hydrogen peroxide produced and can be monitored by flow cytometry. Hydrogen peroxide is cytotoxic, therefore lower levels of hydrogen peroxide production is desirable for cellular viability.

354 In all cases, the three component wound healing composition surpassed the predicted outcomes, clearly demonstrating unpredicted synergy.

Results

1	2	3	4
1 - Control	250	250	0
2 - Fatty Acids (0.5%)	250	230	-20
3 - Sodium Pyruvate (10 mM)	250	490	+240
4 - Vitamin E (50 units)	250	400	+150
5 - Pyruvate & Fatty Acids	250	430	+180
6 - Vitamin E & Fatty Acids	250	200	-50
7 - Pyruvate & Vitamin E	250	290	+40
8 - Pyruvate & Vitamin E & Fatty Acids	250	120	-130

Column 1 shows the different treatment groups.
Column 2 shows the production of H.sub.2 O.sub.2 in control cells (fmol/cell).
Column 3 shows the production of H.sub.2 O.sub.2 after treatment with wound healing components.
Column 4 shows the difference in production of H.sub.2 O.sub.2 from control after the treatment.

355 All comparisons were assessed against the controls, which produced 250 H.sub.2 O.sub.2 fmol/cell. The positive numbers represent H.sub.2 O.sub.2 production in excess of the control and the negative numbers represent H.sub.2 O.sub.2 production below the control. These results are set out in FIG. 8.

Fatty Acids (-20) & Vitamin E (+150) & Pyruvate (+240)
+370 Is The Predicted Three Component Effect
-130 Is The Wound healing composition Actual Effect
500 Is The Difference Between Predicted Effect minus Actual effect (Synergy)
Combination of Paired and Single Ingredients
Pyruvate & Fatty Acids (+180) & vitamin E (+150)
+330 Is The Predicted Three Component Effect
-130 Is The Wound healing composition Actual Effect
460 Is The Difference between Predicted Effect minus Actual Effect (Synergy)
Vitamin E & Fatty Acids (-50) & Pyruvate (+240)
+190 Is The Predicted Three Component Effect
-130 Is The Wound healing composition Actual Effect
320 Is The Difference between Predicted Effect minus Actual Effect (Synergy)
Pyruvate & Vitamin E (+40) & Fatty Acids (-20)
+20 Is The Predicted Three Component Effect
-130 Is The Wound healing composition Actual Effect
150 Is The Difference between Predicted Effect minus Actual Effect (Synergy)

356 In all cases, the three component wound healing composition surpassed the predicted outcomes clearly demonstrating unpredicted synergy.

357 Examples of the Therapeutic Wound Healing Compositions of Embodiment One (I.A-D)

Study 3

358 This study demonstrates a comparison of the wound healing abilities of the therapeutic wound healing compositions of the present invention versus conventional wound healing compositions. The results of this study are illustrated in examples A-D.

359 The wound healing compositions of Examples A-D were prepared having the compositions set out in Table A.

Examples				
Ingredient	A Prep-H .TM.	B	C	D
sodium pyruvate	--	2%	--	--
vitamin E	--	1%	--	--
chicken fat	--	2%	--	--
LYCD	2000 U*	2400 U	2400 U	--
shark liver oil	3%*	3%	3%	--
petrolatum	in	64%	66.5%	68%
mineral oil	amounts	22.53%	25.03%	26.8%
paraffin	totaling	5%	5%	5%
emulsifier	100%	0.2%	0.2%	0.2%

*These components are present in Preparation H

360 Wound healing composition A was commercially available Preparation H.TM.. Wound healing composition B was a petrolatum base formulation containing live yeast cell derivative, shark oil, and a mixture of sodium pyruvate, vitamin E, and chicken fat. Wound healing composition C was a petrolatum base formulation containing live yeast cell derivative and shark oil. Wound healing composition D was a petrolatum base formulation only.

361 Wound healing studies were carried out using hairless mice (SKR-1, Charles River) 6-8 weeks in age. One group of mice were untreated as a control group and were referred to as Example E. In each group there were 6 mice for evaluation at either day 3 or day 7 for a total number of 60 animals in the study. The mice were anesthetized with ether and a midline 3 cm full thickness longitudinal incision was made with a number 10 scalpel blade. Incisions were closed using steel clips at 1 cm intervals. Formulations A-D set out above were applied in a randomized blinded study to the wounds on day 0 at 2 hours following wounding and reapplied at 24 hour intervals during the 7 days of the study. The wounds were examined daily and scored on a basis of 0-5 for closure on each day of the study, with a score of 5 representing the wound best healed.

362 The animals were sacrificed on day 3 and day 7 using cervical dislocation. The dorsal skin including the incision was dissected without the subcutaneous tissue. The skin was placed in neutral buffered formalin and subsequently sectioned and stained with hematoxylin and eosin. The wounds were examined microscopically and representative tissue sections were photographed.

363 On each day of the experiment, the score and rank order of the formulations for closure of wounds and speed of healing were as follows:

B (5)>>D (4)>>C (2)>/=E, Control (2)>A (1)

364 Photographs of the wounded mice on day 4 are set out in FIGS. 9A-9D and 10.

365 FIGS. 9A-9D and 10 show that Formulation B, which was a petrolatum base formulation containing live yeast cell derivative, shark oil, and a mixture of sodium pyruvate, vitamin E, and chicken fat, was a significantly better wound healing agent than the other formulations. These results are supported by the subjective grading of the wound closures and the speed of healing on each day (1-7) of the experiment as well as on the objective histological examination of tissue sections to measure the extent of inflammatory cell infiltrate within the wound and the extent of epithelialization at the wound edges. The final result was that less scar tissue was present at day 7 on the mice treated with Formulation B.

366 Formulation D, which was a white petrolatum formulation only, was judged to be significantly more effective to promote healing than either Formulation C, which was a petrolatum base formulation containing shark liver oil and live yeast cell derivative, or Formulation A, which was Preparation H.TM.. The superior ability of Formulation D over Formulation C to improve healing may result from a delay in the healing process caused when the live yeast cell derivative is depleted and the cells shift to an alternative nutrient source. The presence of the mixture of sodium pyruvate, vitamin E, and chicken fat in Formulation B apparently offsets the depletion of the live yeast cell derivative.

367 Formulation C, which was a petrolatum base formulation containing live yeast cell derivative and shark oil, was judged comparable to the control (untreated wound) in speed of wound closure and extent of healing. Formulation A, which was Preparation H.TM., appeared to be the least effective healing formulation by both subjective grading of wound healing and by objective examination of tissue sections. The superior ability of Formulation D and Formulation C over Formulation A to improve healing may be due to their ability to act as an occlusive wound dressing that prevents transepidermal water loss and thus promotes healing and wound closure. The poor ability of Formulation A to improve healing may be due to the potential cytotoxicity of phenylmercuric nitrate present in Preparation H.TM. as a preservative.

368 These results show that the wound healing compositions of the present invention which comprise a mixture of sodium pyruvate, vitamin E, and chicken fat increase the proliferation and resuscitation rate of mammalian cells. The wound healing compositions mediate low levels of oxygen in the initial stages of healing to suppress oxidative damage and higher levels of oxygen in the later stages of healing to promote collagen formation.

369 II. Augmented Wound Healing Compositions A. Embodiment Two (I.A-D+M)

370 In Embodiment Two (II), the therapeutic wound healing compositions of Embodiment One (I.A-D) are combined with a medicament (M) which is useful for treating injured mammalian cells to form augmented wound healing compositions (I.A-D+M) having an enhanced ability to prevent and reduce injury to mammalian cells and further increase the resuscitation rate of injured mammalian cells. The tissue damage associated with many diseases and conditions such as autoimmune disease and benign and malignant skin growths is believed to be caused by the production of cellular produced active oxygen species. Combination of the therapeutic wound healing compositions of the present invention and medicaments useful for treating such diseases and conditions may suppress such reactive oxygen-linked tissue injury.

371 For example, the therapeutic wound healing compositions may be used in topical augmented wound healing compositions in combination with medicaments useful for treating wounds such as immunostimulating agents (Betafectin.TM.), antiviral agents, antikeratolytic agents, anti-inflammatory agents, antifungal agents, tretinoin, sunscreen agents, dermatological agents, topical antihistamine agents, antibacterial agents, bioadhesive agents, respiratory bursting inhibitors (lactic acid, adenosine), inhibitors of prostaglandin synthesis (ibuprofen, aspirin, indomethacin, meclofenomic acid, retinoic acid, padimate O,

meclomen, oxybenzone), steroidal anti-inflammatory agents (corticosteroids including synthetic analogs), antimicrobial agents (neosporin ointment, silvadine), antiseptic agents, anesthetic agents (pramoxine hydrochloride, lidocaine, benzocaine), cell nutrient media, burn relief medications, sun burn medications, acne preparations, insect bite and sting medications, wound cleansers, wound dressings, scar reducing agents (vitamin E), and the like, and mixtures thereof, to further enhance the proliferation and resuscitation rate of mammalian cells. Preferably, the medicament useful for treating wounds is selected from the group consisting of immunostimulating agents, antiviral agents, antikeratolytic agents, anti-inflammatory agents, antifungal agents, tretinoin, sunscreen agents, dermatological agents, topical antihistamine agents, antibacterial agents, bioadhesive agents, respiratory bursting inhibitors, inhibitors of prostaglandin synthesis, antimicrobial agents, cell nutrient media, scar reducing agents, and mixtures thereof. More preferably, the medicament useful for treating wounds is selected from the group consisting of immunostimulating agents, antiviral agents, antikeratolytic agents, anti-inflammatory agents, antifungal agents, acne treating agents, sunscreen agents, dermatological agents, antihistamine agents, antibacterial agents, bioadhesive agents, and mixtures thereof.

372 The therapeutic wound healing compositions may also be used in ingestible augmented wound healing compositions in combination with medicaments used to treat injured mammalian cells such as stroke medications; autoimmune disease medications; arthritis medications; ulcer medications; cancer medications (cytotoxic agents); heart medication to improve regional ventricular function and restore normal heart rate and pressure functions; lung medication to repair injured tissue; liver medication to suppress lipogenesis of alcoholic origin and prevent hepatic steatosis; kidney medication to suppress urinary calculi (kidney stones); detoxification medication to antagonize heavy metal poisoning, cyanide poisoning, sodium sulfide poisoning, other types of poisoning; and reduce and neutralize the production of oxygen radicals which produces injury to tissue, to protect and further enhance the resuscitation rate of the injured mammalian cells.

373 A cell nutrient medium provides a complete diet of nutrients necessary for wound healing. The cell nutrient medium may be derived from animal, plant, and yeast sources. Typical cell nutrient media includes live yeast cell derivative, Eagles medium, and artificial serum. A preferred cell nutrient medium is live yeast cell derivative. Live yeast cell derivative supplies skin respiratory factor which acts by increasing the oxygen uptake of dermal tissues and facilitates collagen formation. Live yeast cell derivative generally contains numerous amino acids for collagen formation, mono- and disaccharides as carbon sources, vitamins, minerals, phosphorous containing compounds, nucleosides, nucleotides, and salts. In general, the amino acids present in live yeast cell derivative include aspartic acid, glutamic acid. histidine, serine, glycine, alanine, arginine, tyrosine, valine, methionine, isoleucine, leucine, phenylalanine, and lysine. The coenzymes present in live yeast cell derivative include vitamin A, vitamin E, vitamin D.sub.3, folic acid, pantothenic acid, niacinamide, vitamin B.sub.1, vitamin B.sub.2, vitamin B.sub.6, and vitamin B.sub.12. The cofactor type minerals present in live yeast cell derivative include calcium, copper, iron, magnesium, zinc, and phosphorus. A preferred tissue respiratory factor is Biodynes.RTM. TRF, commercially available from Brooks Industries, Inc., South Plainfield, N.J. In general, the cell nutrient medium will be present in the therapeutic composition in an amount from about 0.01% to about 5%, preferably from about 0.1% to about 1%, and more preferably from about 0.2% to about 0.4%, by weight of the therapeutic composition.

374 Infected wounds are generally treated with an antibiotic agent, an antifungal agent, or an antiviral agent to kill the infecting organism. But these agents do not facilitate healing. Combination of the wound healing compositions of Embodiment One (I.A-D) with an antibiotic agent, an antifungal agent, or an antiviral agent would provide an augmented wound healing composition (I.A-D+M) which would kill the infecting organism and facilitate healing. The wound healing compositions can be used in combination with (a) an antibacterial agent; (b) an antifungal agent for athlete's foot (Clotrimazole), infected toes, jock

itch, and vaginal infections; (c) an antiviral agent for cold sores (Acyclovir), genital lesions, and HIV lesions (AZT) (heals and decreases duration and severity of viral lesions).

375 Damage from UV light can be avoided through the use of antioxidants. Combination of the wound healing compositions of Embodiment One (I.A-D) with an anti-inflammatory agent would provide an augmented wound healing composition (I.A-D+M) which would repair and reduce the erythema caused by the inflammatory responses to overexposure to UV light. The augmented wound healing compositions can be used for UV protection with SPF agents, UV repair (protects and repairs damaged skin), and antiaging (protects skin from UV damage and aging).

376 Certain therapeutic drugs are known to have irritating side effects. Combination of the wound healing compositions of Embodiment One (I.A-D) with a drugs that has irritating side effects would provide an augmented wound healing composition (I.A-D+M) which would have therapeutic value. For example, the wound healing compositions can be used in combination with Retin A (reduces irritation of actives) and Vitamin D and analogs (reduces irritation of actives).

377 Lips are often protected from UV damage with lip balms that contain moisturizing agents and antioxidants. Combination of the wound healing compositions of Embodiment One (I.A-D) with moisturizing agents would provide an augmented wound healing composition (I.A-D+M) which would provide a healing benefit for dry cracking lips. The augmented wound healing compositions can be used for lip protection (provides protection and repair from UV light, cold etc), lip moisturizing (provides a moisture barrier), and mouth sores in combination with an antiseptic (heals sores and kills the germs).

378 Gingivitis is initiated by supragingival plaque. Uncontrolled supragingival plaque releases toxins and microbial products that attack the gingiva and result in inflammation of the gingival tissues. Inflammation in the connective tissues results in pocket formation and may ultimately result in periodontitis. Oxygen radicals at the site of infection can cause tissue damage. Combination of the wound healing compositions of Embodiment One (I.A-D) with anti-inflammatory agents or antimicrobial agents would provide an augmented wound healing composition (I.A-D+M) which may help decrease inflammation and improve healing of damaged tissues. The augmented wound healing composition can be used in tooth paste (enhances healing of bleeding gums); mouth washes (helps maintain healthy gums); bioadhesive films (provides long term healing agents for injured gums); and products to heal cuts from orthodontic appliances.

379 Sore throats can be caused by many factors including simple irritation due to shouting, infections, smoking, or eating the wrong foods. Combination of the wound healing compositions of Embodiment One (I.A-D) with a throat lozenge would provide an augmented wound healing composition (I.A-D+M) which would soothe and heal the throat with and without antimicrobial agents. The wound healing composition can be used for irritated sore throats (soothes and heals inflamed sore throats); in combination with antivirals (soothes and heals inflamed tissues); and in combination with antimicrobials (kills the infection, heals the sore throat faster).

380 Combination of the wound healing compositions of Embodiment One (I.A-D) with women's hygeine products with and without drugs or antimicrobials would provide an augmented wound healing composition (I.A-D+M) which would heal the irritated skin and tissues. The augmented wound healing composition can be used in vaginal products with and without antimicrobials (reduces vaginal irritation); and post pregnancy products, i.e., breast and stomach creams (reduces stretch marks).

381 In a specific embodiment, the invention is directed to an augmented wound healing composition (Embodiment Two (I.A-D+M)) having an enhanced ability to prevent and reduce injury to mammalian cells which comprises:

382 (A) a therapeutic wound healing composition selected from the group of consisting of:

383 (I.A) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

384 (b) an antioxidant; and

385 (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells;

386 (I.B) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

387 (b) lactate selected from the group consisting of lactic acid, pharmaceutically acceptable salts of lactic acid, and mixtures thereof; and

388 (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells;

389 (I.C) (a) an antioxidant; and

390 (b) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells;

391 (I.C) (a) lactate selected from the group consisting of lactic acid, pharmaceutically acceptable salts of lactic acid, and mixtures thereof;

392 (b) an antioxidant; and

393 (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells; and

394 (B) a medicament useful for treating injured mammalian cells.

395 In a preferred embodiment, the augmented wound healing composition comprises the therapeutic wound healing compositions of the present invention and a medicament useful for treating injured mammalian cells selected from the group consisting of anti-inflammatories and wound cleansers and wound dressings. In a more preferred embodiment, the augmented wound healing composition comprises a medicament selected from the group consisting of wound cleansers and wound dressings.

396 In a preferred embodiment, the invention is directed to an augmented wound healing composition (I.A+M) having an enhanced ability to prevent and reduce injury to mammalian cells which comprises:

397 (A) a therapeutic wound healing composition which comprises:

398 (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

399 (b) an antioxidant; and

400 (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the resuscitation of injured mammalian cells; and

401 (B) a medicament useful for treating injured mammalian cells.

402 B. Methods For Making the Augmented Wound Healing Compositions of Embodiment Two (I.A-D+M)

403 The present invention extends to methods for making the therapeutic augmented

wound healing compositions (I.A-D+M). In general, a therapeutic augmented wound healing composition is made by forming an admixture of the wound healing components of Embodiment One (I.A-D) and a medicament which is useful for treating injured mammalian cells. In a first aspect of Embodiment Two (I.A+M), an augmented wound healing therapeutic composition is made by forming an admixture of a medicament which is useful for treating injured mammalian cells and a wound healing composition comprising (a) a pyruvate, (b) an antioxidant, and (c) a mixture of saturated and unsaturated fatty acids. In a second aspect of Embodiment Two (I.B+M), an augmented wound healing therapeutic composition is made by forming an admixture of a medicament which is useful for treating injured mammalian cells and a wound healing composition comprising (a) a pyruvate, (b) a lactate, and (c) a mixture of saturated and unsaturated fatty acids. In a third aspect of Embodiment Two (I.C+M), an augmented wound healing therapeutic composition is made by forming an admixture of a medicament which is useful for treating injured mammalian cells and a wound healing composition comprising (a) an antioxidant, and (b) a mixture of saturated and unsaturated fatty acids. In a fourth aspect of Embodiment Two (I.D+M), an augmented wound healing therapeutic composition is made by forming an admixture of a medicament which is useful for treating injured mammalian cells and a wound healing composition comprising (a) a lactate, (b) an antioxidant, and (c) a mixture of saturated and unsaturated fatty acids.

404 In a preferred embodiment, the invention is directed to a method for preparing a therapeutic augmented wound healing composition (I.A+M) which comprises the steps of admixing the following ingredients:

405 (A) a wound healing composition which comprises:

406 (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

407 (b) an antioxidant; and

408 (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells; and

409 (B) a therapeutically effective amount of a medicament which is useful for treating injured mammalian cells.

410 C. Methods For Employing the Augmented Wound Healing Compositions of Embodiment Two (I.A-D+M)

411 The present invention extends to methods for employing the therapeutic augmented wound healing compositions (I.A-D+M). In general, an augmented wound healing composition is employed by contacting the composition with a wound. In a preferred embodiment, the invention is directed to a method for healing a wound in a mammal with an augmented wound healing composition (I.A+M) which comprises the steps of:

412 (A) providing an augmented wound healing composition which comprises:

413 (1) a therapeutic wound healing composition which comprises:

414 (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

415 (b) an antioxidant; and

416 (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells; and

417 (2) a medicament useful for treating wounds; and

418 (B) contacting the augmented wound healing composition with the wound.

419 The types of wounds which may be healed using the compositions of the present invention are those which result from an injury which causes epidermal damage, chronic ulcers, gastric ulcers, burns, donor site wounds, and the like. Such wounds include ophthalmic wounds, such as those which result from corneal ulcers, radialkeratotomy, corneal transplants, epikeratophakia and other surgically induced wounds in the eye, and cutaneous wounds such as burn wounds, donor site wounds from skin transplants and ulcers (cutaneous, decubitus, venous stasis, and diabetic). In addition, dermatological wounds such as psoriasis, sunburn, and skin rashes may also be treated with the compositions of the present invention. The compositions may be applied to the wound site either topically or internally depending on the type of wound.

420 Methods for increasing the rate of wound healing comprise contacting the wound healing composition with the wound to increase the healing rate of the wound. Preferably, the method comprises topically administering the compositions of the present invention directly to a wound site. The composition is maintained in contact with the wound for a period of time sufficient to increase the rate of cell growth at the wound site.

421 D. Formulations of the Augmented Wound Healing Compositions of Embodiment Two (I.A-D+M)

422 Once prepared, the inventive therapeutic augmented wound healing compositions may be stored for future use or may be formulated in effective amounts with pharmaceutically acceptable carriers such as pharmaceutical appliances and topical vehicles (oral and non-oral) to prepare a wide variety of pharmaceutical compositions. The pharmaceutically acceptable carriers which may be employed and the methods used to prepare the pharmaceutical compositions have been described above in connection with the formulations of the wound healing compositions of Embodiment One (I.A-D).

423 In a specific embodiment, the invention is directed to an augmented wound healing pharmaceutical composition which comprises:

424 (A) a therapeutic augmented wound healing composition which comprises:

425 (1) a wound healing composition which comprises:

426 (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

427 (b) an antioxidant; and

428 (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and

429 (2) a therapeutically effective amount of a medicament useful for treating wounds; andresuscitation of mammalian cells; and

430 (B) a pharmaceutically acceptable carrier selected from the group consisting of pharmaceutical appliances, bioadhesives, and occlusive vehicles.

431 In another specific embodiment, the invention is directed to a method for preparing an augmented wound healing pharmaceutical composition for increasing the proliferation and resuscitation rate of mammalian cells, which comprises the steps of:

432 (A) providing a therapeutically effective amount of an augmented wound healing composition which comprises:

433 (1) a medicament useful for treating wounds; and

434 (2) a wound healing composition comprising: